

FORM PTO-1390
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER

11184.00002

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/869777

INTERNATIONAL APPLICATION NO.
PCT/AU00/00003INTERNATIONAL FILING DATE
5 January 2000PRIORITY DATE CLAIMED
5 January 1999

TITLE OF INVENTION

CRYPTATE COMPOUNDS AND METHODS FOR DIAGNOSIS AND THERAPY

APPLICANT(S) FOR DO/EO/US

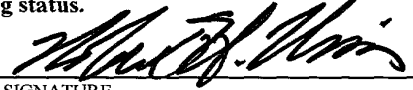
SMITH, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
 - International Publication WO 00/40585, with International Search Report and Amended Claims (57 sheets)
 - International Preliminary Examination Report (6 sheets)
 - PCT Request (4 sheets)

U.S. APPLICATION NO. (if known) 097869777 PCT/AU00/00003		INTERNATIONAL APPLICATION NO PCT/AU00/00003		ATTORNEY'S DOCKET NUMBER 11184.00002	
21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 1,000.00	
				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	23 - 20 =	3	x \$18.00	\$	54.00
Independent claims	2 - 3 =	0	x \$80.00	\$	0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+	\$270.00
TOTAL OF ABOVE CALCULATIONS =				\$	1,184.00
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	\$ 592.00
SUBTOTAL =				\$	592.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	592.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				\$	592.00
				Amount to be refunded:	\$
				charged:	\$
a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed. b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>01-0850</u> in the amount of \$ <u>592.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>01-0850</u> . A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Robert H. Resis BANNER & WITCOFF, LTD Ten South Wacker Drive, Suite 3000 Chicago, IL 60606-7407					
				 SIGNATURE	
				ROBERT H. RESIS NAME	
				32,168 REGISTRATION NUMBER	

Cryptate Compounds and Methods for Diagnosis and Therapy

Technical Field

The present invention relates to cryptate compounds useful as chelating agents. In particular, the present invention relates to functionalised derivatives of certain cryptate compounds. These functionalised derivatives are suitable for use in radiolabelling and similar applications. The present invention also relates to a method for diagnosis or therapy of a disease utilising functionalised derivatives of cryptate compounds.

Background of the Invention

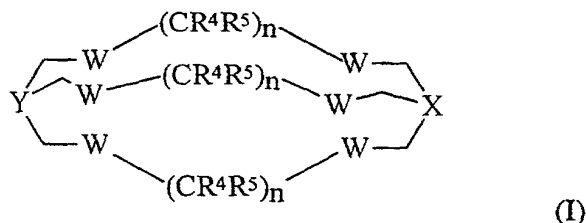
Radiolabelled compounds are useful as radiopharmaceuticals, imaging agents, or the like which are especially useful for but not limited to the diagnosis and therapy of diseases including cancer.

Known radiolabelled compounds suffer from the disadvantage that, in use, the radiolabelled nuclide can become detached from the carrier compound thereby leading to problems and potential complications in diagnostic and therapeutic applications. Further, the known radiopharmaceuticals tend to be non-specific in their biodistribution throughout a subject.

The present invention seeks to ameliorate the stated disadvantages of the prior art by providing compounds which are capable of being radiolabelled more expeditiously, specifically target a localised area of tissue or an organ in a subject and which are more stable than the prior art compounds and less toxic. Further, the compounds of the present invention are typically suitable for use in pharmaceutical formulations. It is a further typical object of the present invention to provide a method of diagnosis or therapy of disease in a subject.

Summary of the Invention

In accordance with a first embodiment of the present invention, there is provided a compound which is capable of being radiolabelled of general formula (I) which is as follows:



in which n represents an integer from 2 to 4,

where each R^4 and R^5 is independently selected from -H, CH_3 , COOH , NO_2 , CH_2OH , H_2PO_4 , HSO_3 , CN , C(=O)NH_2 and CHO ;

X and Y are the same or different and are selected from the group of C-R, N, P and C-Z in which R is selected from hydrogen, halogen, hydroxyl, nitro, nitroso, amino, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl,

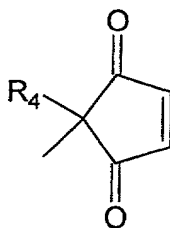
cyano, $-\text{COOR}'$, COCOOR' , $\text{NH-COCH}_2\text{Br}$, $-\text{NH-CO-CH=CH-COOR}'$ in which R' is a hydrogen atom or alkyl group;

W is selected from the group of NH , S and O ; and

Z is a functionalised linkage group which is capable of binding said compound of formula (I) to a molecular recognition unit and wherein at least one of X and Y is C-Z ; or a pharmaceutically acceptable salt thereof.

It is to be understood that throughout this specification, the term "molecular recognition unit" includes an antibody, protein, peptide, carbohydrate, nucleic acid, oligonucleotide, oligosaccharide, liposome, or other molecule which can form part of a specific binding pair.

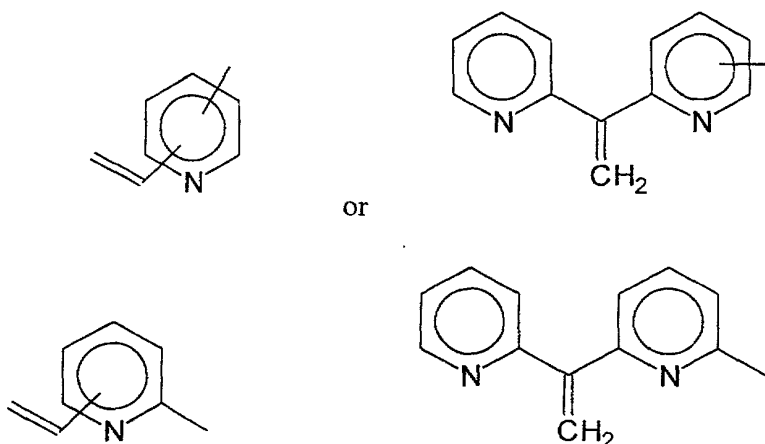
In one form of the compound of Formula (I), the functionalised linkage group Z is selected from the group of halogen or other leaving group, nitro, nitroso, imide, dione of the formula,



vinyl group of formula $\text{Het}^1\text{-C(Het}^2\text{)=CH}_2$ (where Het^1 and Het^2 are the same or different and is each a nitrogen containing heterocyclic group or Het^1 is a nitrogen containing heterocyclic group and Het^2 is H), $-\text{C(=NH)OR}^2$, NCO , NCS , COR'' , COOR' , SR^2 , NHN^2R^3 , $\text{NHCONHN}^2\text{R}^3$, $\text{NHCSNHN}^2\text{R}^3$, CONR^2 , OR^2 , NR^2R^3 , $(\text{CH}_2)_p\text{R}^1$, $(\text{CH}_2)_p\text{ArR}^1$, $(\text{CH}_2\text{O})_p\text{CH}_2\text{R}^1$, $(\text{CH}_2\text{OCH}_2\text{O})_q\text{ArR}^1$, $(\text{CHCH})_r\text{R}^1$, $(\text{CHCH})_r\text{ArR}^1$ where R^2 and R^3 are the same or different and are independently selected from H , $(\text{CH}_2)_p\text{R}^1$, $(\text{CH}_2)_p\text{ArR}^1$, $(\text{CH}_2\text{O})_p\text{CH}_2\text{R}^1$, $-(\text{CH}_2\text{OCH}_2\text{O})_q\text{ArR}^1$, $(\text{CHCH})_r\text{R}^1$, $(\text{CHCH})_r\text{ArR}^1$ and where R^1 is selected from SH , OH , NH_2 , COOH , NCS , $-\text{N=N}$, or $-\text{C(=NH)OCH}_3$, COR'' , where R'' is H , halogen, N_3 , alkoxy, OAr , imidyloxy, imidazoyloxy, alkyl, or alkyl substituted with a halogen or other leaving group, where p is an integer from 1 to 20, more typically 1 to 10, still more typically 1 to 4, even more typically 1 to 2 and yet more typically 1; q is an integer from 1 to 20, more typically 1 to 10, still more typically 1 to 4, even more typically 1 to 2 and yet more typically 1; r is an integer from 1 to 4, more typically 1 or 2, still more typically 1; and Ar is optionally substituted aryl or optionally substituted aralkyl, provided that when one of X and Y is selected from C-NO_2 , C-OH , C-Cl , C-CH_3 or C-NH_2 then the other X or Y substituent cannot be selected from C-NO_2 , C-OH , C-Cl or C-NH_2 . In moieties of formula $(\text{CH}_2)_p\text{R}^1$, $(\text{CH}_2)_p\text{ArR}^1$, one or more methylene groups may also be replaced with O , S , NH or carbonyl, for example C(O)R^1 , $\text{CH}_2\text{C(O)R}^1$, NHCH_2R^1 , NHC(O)R^1 , CH_2OR^1 , $\text{CH}_2\text{C(O)NHR}^1$, and the like.

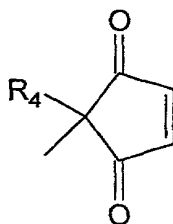
3

Typically in the compound of Formula (I), the functionalised linkage group Z of the compound of Formula (I) is selected from the group of halogen, maleimide, a vinyl pyridyl group of formula



especially

or a dione of formula



;

NR²R³ where R² and R³ are the same or different and are independently selected from H, (CH₂)_pR¹, (CH₂)_pArR¹, (CH₂O)_pCH₂R¹, -(CH₂OCH₂O)_qArR¹, -(CHCH)_rR¹, (CHCH)_rArR¹ and where R¹ is selected from NH₂, COOH, NCS, NCO, -N=N-, or -C(=NH)OCH₃, COR" group where R" is H, halogen, alkyl, or alkyl substituted with a halogen or other leaving group, where p is an integer from 1 to 20, more typically 1 to 10, still more typically 1 to 4, even more typically 1 to 2 and yet more typically 1; q is an integer from 1 to 20, more typically 1 to 10, still more typically 1 to 4, even more typically 1 to 2 and yet more typically 1; r is an integer from 1 to 4, more typically 1 or 2, still more typically 1; and Ar is optionally substituted aryl or optionally substituted aralkyl, provided that at least one of R² and R³ is other than hydrogen, and wherein, in moieties of formula (CH₂)_pR¹, (CH₂)_pArR¹, one or more methylene groups may also be replaced with O, S, NH or carbonyl, for example C(O)R¹, CH₂C(O)R¹, NHCH₂R¹, NHC(O)R¹, OR¹, SR¹, CH₂OR¹, CH₂C(O)NHR¹, and the like.

More typically in a compound of Formula (I), each R⁴ and R⁵ is H; W is NH; n is 2 to 4, more typically 2 or 3, still more typically 2; Z is selected from halogen and NR²R³ where R² and R³ are the same or different and are independently selected from H, (CH₂)_pR¹, (CH₂)_pArR¹, provided that at least one of R² and R³ is other than H; R¹ is

selected from NH_2 , COOH , NCS , NHCOCH_2Br and COR'' where R'' is halogen, typically Br; and p is an integer from 1 to 4, more typically 1 to 2 and still more typically 1. Yet more typically, R^1 is NH_2 . Typically, R is amino, nitro, hydroxy or halogen and still more typically R is amino.

As used herein, the term "aryl" refers to single, polynuclear, conjugated and fused residues of aromatic hydrocarbons or aromatic heterocyclic ring systems. Examples of such groups are phenyl, biphenyl, terphenyl, quaterphenyl, naphthyl, tetrahydronaphthyl, anthracenyl, dihydroanthracenyl, benzanthracenyl, dibenzanthracenyl, phenanthracenyl, fluorenyl, pyrenyl, indenyl, azulenyl, chrysenyl, pyridyl, 4-phenylpyridyl, 3-phenylpyridyl, thienyl, furyl, pyrrol, indolyl, pyridazinyl, pyrazolyl, pyrazinyl, thiazolyl, pyrimidinyl, quinolyl, isoquinolyl, benzofuranyl, benzothienyl, purinyl, quinazolinyl, phenazinyl, acridinyl, benzoxazolyl, benzothiazolyl, heteroaryl, pyridine and Het-CH=CH_2 and the like. Typically aryl is phenyl, pyridyl, naphthyl, anthracenyl or the like, and heteroaryl is typically pyridine and Het-CH=CH_2 . Still more typically, aryl is phenyl.

As used herein, the term "aralkyl" refers to alkyl groups substituted with one or more aryl groups as previously defined. Examples of such groups are benzyl, 2-phenylethyl and 1-phenylethyl.

As used herein, the term "optionally substituted" means that the moiety described as optionally substituted may carry one or more substituents selected from amino, halogen, hydroxy, mercapto, nitro, cyano, thiocyno, alkyl, alkoxy, halogenoalkyl, acyl, acylamino, acyloxy, carboxyl, alkoxycarboxyl, carbamoyl, pyridoylamino, carboxyalkyl-carbamoyl, N-carboxyalkylcarbamoyl, sulpho, sulphamoyl, mono- or dialkylated or phenylated sulphamoyl which can carry one or more alkyl substituents, alkylsulphonyl, alkoxysulphonyl, optionally hydroxy-containing phenylsulphonyl or phenoxy sulphonyl.

In another form of the compound of Formula (I), the Z group of said compound of Formula (I) is selected from the group of NR^2R^3 where R^2 and R^3 together with the nitrogen atom to which they are attached form a substituted saturated or unsaturated 3 to 8 membered ring optionally containing one or more additional heteroatoms O, S or N and wherein there is at least one substituent capable of binding said compound of Formula (I) with a molecular recognition unit.

In accordance with a second embodiment of the present invention, there is also provided a compound of Formula (I) as described in the first embodiment of the present invention which is complexed with a metal ion.

The metal ion is typically selected from ^{64}Cu , ^{67}Cu , $^{99\text{m}}\text{Tc}$, Ga, In, Co, Re, Fe, Au, Ag, Rh, Pt, Bi, Cr, W, Mo, Ni, V, Pb, Ir, Pt, Zn, Cd, Mn, Ru, Pd, Hg, Ti, Tl, Sn, Zr, and the lanthanide group of elements in the Periodic Table such as Sm, Ho, Gd, Tb, Sc, Y, and the actinides.

The metal ion is further typically a radionuclide selected from the group of ^{64}Cu , ^{67}Cu , $^{99\text{m}}\text{Tc}$, and radionuclides of In(III), Ga(III), Fe(III), Cu(II), Ti(IV) and other radionuclides from the Lanthanides, Re, Sm, Ho, and Y.

In accordance with a third embodiment of the present invention, there is also provided a pharmaceutical formulation comprising a compound of Formula (I) as described in the first embodiment of the present invention or a metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

In accordance with a fourth embodiment of the present invention, there is also provided a diagnostic formulation comprising a compound of Formula (I) as described in the first embodiment of the present invention or a metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof and a reducing agent in a pharmaceutically acceptable carrier.

In accordance with a fifth embodiment of the present invention, there is also provided a method of diagnosis or therapy in a subject comprising administering to the subject a diagnostically or therapeutically effective amount of a compound of Formula (I) as described in the first embodiment of the present invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof.

In accordance with a sixth embodiment of the present invention, there is also provided a use of a compound of Formula (I) or a metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof in the preparation of a medicament for diagnosis or therapy of disease in a subject.

In accordance with a seventh embodiment of the present invention, there is also provided a compound of Formula (I) as described in the first embodiment of the present invention or a metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof when used in the diagnosis or therapy of disease in a subject.

In accordance with an eighth embodiment of the present invention, there is also provided a conjugate compound comprising at least one compound of Formula (I) as described in the first embodiment of the present invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof bonded to at least one molecular recognition unit comprising an antibody, protein, peptide, carbohydrate, oligonucleotide, oligosaccharide, liposome or the like.

In accordance with a ninth embodiment of the present invention, there is provided a method of diagnosis or therapy in a subject comprising administering to the subject a diagnostically or therapeutically effective amount of a conjugate compound as described in the eighth embodiment of the present invention.

In accordance with a tenth embodiment of the present invention, there is provided a use of a conjugate compound as described in the eighth embodiment of the present invention in the preparation of a medicament for diagnosis or therapy of disease in a subject.

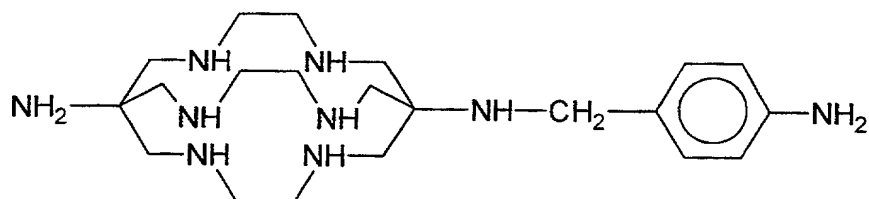
In accordance with an eleventh embodiment of the present invention, there is provided a conjugate compound as described in the eighth embodiment of the present invention when used in the diagnosis or therapy of disease in a subject.

In accordance with a twelfth embodiment of the present invention, there is provided a method of imaging a subject comprising introducing a compound of Formula (I) or a metal complex, radiolabelled complex, conjugate compound or pharmaceutically acceptable salt thereof to a subject.

In accordance with a thirteenth embodiment of the present invention, there is provided a use of a compound of Formula (I) or a metal complex, radiolabelled complex, conjugate compound or pharmaceutically acceptable salt thereof in the preparation of a medicament for imaging in a subject.

In accordance with a fourteenth embodiment of the present invention, there is provided a compound of Formula (I) or a metal complex, radiolabelled complex, conjugate compound or pharmaceutically acceptable salt thereof when used in imaging in a subject.

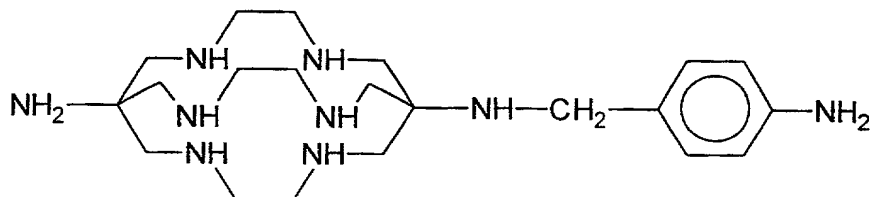
In accordance with a fifteenth embodiment of the present invention, there is provided a compound of Formula (I) having the following structure:



Detailed Description Of The Invention

The compounds of Formula (I) typically comprise those compounds where n represents an integer from 2 to 4, more typically 2 or 3, still more typically 2. Typically, one of X and Y is a $C-Z$ group where Z is typically NR^2R^3 where R^2 and R^3 are the same or different and are selected from H , $(CH_2)_pR^1$ and $(CH_2)_pArR^1$, where R^1 is as previously defined, and p is 1 to 4, more typically 1 to 2 and still more typically 1, provided that at least one of R^2 and R^3 is other than hydrogen. Usually, one of X is a $C-Z$ group and the other is a group $C-R$, where R is as previously defined, typically amino, lower alkyl, nitro, hydroxy or halogen. Generally, R^1 is NH_2 and p is 1.

An example of a compound of Formula (I) is:



The R^1 group in a compound of Formula (I) may provide a point of attachment of a compound of Formula (I) to a molecular recognition unit.

The molecular recognition unit is typically an antibody, protein, peptide, oligonucleotide, oligosaccharide. In particular, the molecular recognition unit is typically an antibody and more typically a monoclonal antibody.

Thus, compounds of Formula (I) provide a method of attachment of radionuclide metal ions such as In(III), Ga(III), Fe(III), Tc(IV) or Tc(V), Re(VII), Cu(II), Ti(IV), other radionuclides from the Lanthanides, Rhenium, Samarium, Holmium, Yttrium and the like to molecular recognition units such as monoclonal antibodies, receptor specific proteins, peptides or oligonucleotides for *in vivo* imaging and therapy.

The compounds of general Formula (I) are typically prepared by attachment of a functional linking group to a suitable cryptate. Methods of synthesis of cryptates useful as precursors to compounds of Formula (I) in which W is NH are described in United States Patent No. 4,497,737 in the name of Sargeson *et al*, the disclosure of which is incorporated herein by reference. Other cryptates where W is S or O may be prepared by analogous methods. Sargeson *et al* describe synthesis of metal cage "cryptate" compounds by the condensation of a tris-(diamine) metal ion complex as described at column 3 lines 30 to 35 with formaldehyde and an appropriate nucleophile. In order to obtain the compounds of Formula (I), an appropriate nucleophile is selected so as to obtain the desired functionalised linkage group Z as defined in Formula (I). In particular, reference is made to column 4 lines 17 to 27 of United States Patent No. 4,497,737. Alternatively, a functionalised linkage group Z may be attached to a functional group of a cryptate prepared by the methods taught by Sargeson *et al* (for example see Example 9 at column 9 line 65 to column 12 line 10 of Sargeson *et al*) by standard synthetic techniques. If necessary, a protecting group may be introduced into the cryptate structure to protect latent functionality for the desired functionalised linkage group Z as defined in Formula (I) during synthesis of the desired cryptate precursor. Suitable protecting groups are described, for example in Greene, T.W., *Protective Groups in Organic Synthesis* (John Wiley & Sons, New York, 1981) and McOmie, J.F.W., *Protective Groups in Organic Chemistry* (Plenum Press, London, 1973).

For example, compounds of Formula (I) where said Z group comprises a mono- or di-substituted amino group and where the substituent is optionally substituted alkyl, are readily prepared by treating the amino compound with the appropriate halo-substituted alkyl. Typically, the compound of Formula (I) where Z is -NH-CH₂-CH₂-NH₂ can be prepared by treating a compound of Formula (I) where R is NH₂ with BrCH₂CH₂NH₂ in the presence of NaHCO₃ or the like with suitable protection.

Compounds of formula (I) where R¹ is -NCS may be prepared by reacting the amino compound with thiophosgene (see WO87/12631), Kozak *et al.*, Cancer Res. 49, 2639 (1989). Substituted acid halide compounds are produced by reacting a compound of formula (I) where R is NH₂ with BrCH₂COBr at 4°C according to the procedure of C J Mathias *et al.*, Bioconjugate Chem., 1, 204 (1990). Compounds with an electrophilic moiety can also be prepared by methods known in the art, such as in ACC Chem. Res. 17 202-209 (1984). Compounds with active esters (CH₃)_p-C(O)-X may be formed by the procedures of Bodanszky M, *The Peptide. Analysis Synthesis Biology*, Ed. E. Gross and J Meienhofer, Vol 1. pp 105-196, Academic Press, Inc., Orlando, FL. (1979) and Bodanszky M, *Principles of Peptide Synthesis*, pp 9-58, Springer-Verlag, New York, (1984). Other compounds of

formula (I) may be prepared from such compounds by standard procedures such as described in Modern Synthetic Reactions, H O House, 2nd Edition, Benjamin, Inc. Philippines, 1972.

In a typical embodiment, compounds of formula (I) in which X or Y is C-Z where the group Z is a group $\text{NH}(\text{CH}_2)_p\text{R}^1$ or $\text{NH}(\text{CH}_2)_p\text{ArR}^1$ may be prepared by a Schiff Base condensation reaction of a compound of formula (I) (or a metal complex thereof) in which X or Y is NH_2 with an aldehyde of formula $\text{HC}(\text{O})(\text{CH}_2)_{p-1}\text{R}^1$ or $\text{HC}(\text{O})(\text{CH}_2)_{p-1}\text{ArR}^1$. (Still typically, the Schiff Base condensation reaction is most appropriately conducted between a copper complex of compounds of Formula (I) in which X or Y is NH_2 with an aldehyde of formula $\text{HC}(\text{O})(\text{CH}_2)_{p-1}\text{R}^1$ or $\text{HC}(\text{O})(\text{CH}_2)_{p-1}\text{ArR}^1$.) In one particular form of this embodiment, a compound of formula (I) is obtained by the reaction of nitrobenzaldehyde with the copper complex of an aminocryptate such as described by Sargeson *et al.* The reaction is typically performed in an inert-gas atmosphere and in the presence of solvent and diluents typically inert to the reactants. Suitable solvents comprise aliphatic, aromatic, or halogenated hydrocarbons such as benzene, toluene, xylenes; chlorobenzene, chloroform, methylene chloride, ethylene chloride; ethers and ethereal compounds such as dialkyl ether, ethylene glycol mono or -dialkyl ether, THF, dioxane; alkanols such as methanol, ethanol, n-propanol, isopropanol; ketones such as acetone, methyl ethyl ketone; nitriles such as acetonitrile or 2-methoxypropionitrile; N,N-dialkylated amides such as dimethylformamide; dimethylsulphoxide, tetramethylurea; as well as mixtures of these solvents with each other. If the amine or a salt thereof is soluble in water, then the reaction medium may be water at low temperature. The compounds of formula (I) may be converted to pharmaceutically acceptable salts by way of recognised procedures.

Typically, for medical use salts of the compounds of the present invention will be pharmaceutically acceptable salts; although other salts may be used in the preparation of the inventive compound or of the pharmaceutically acceptable salt thereof. By pharmaceutically acceptable salt is meant those salts which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

Suitable pharmaceutically acceptable salts of the compounds of the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulphuric acid, methanesulphonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid. Suitable pharmaceutically acceptable salts of the compounds of the present invention therefore include acid addition salts.

For example, S. M. Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66:1-19. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Representative acid addition salts

include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphersulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pantoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

The radiolabelling of compounds of formula (I), and salts thereof can be accomplished by using procedures recognised in the art. For example, radiolabelling of the chelator with ^{67}Cu can be achieved by adding copper in an aqueous acetate solution to a compound of formula (I) in an aqueous solution and incubating at room temperature.

Alternatively, the radiolabelling of a compound of formula (I) with technetium, for example, may be achieved by adding a reducing agent such as stannous salts typically stannous chloride, to an aqueous solution of a compound of formula (I), followed by reaction with aqueous sodium pertechnetate solution ($\text{Na}^{99\text{m}}\text{TcO}_4$). The order of mixing these three components is believed not to be critical. However, typically the reducing agent is added to the chelator of formula (I). Other suitable reducing agents comprise alkali metal dithionites such as sodium dithionite, sodium borohydride, hydrochloric acid, hydrobromic acid, other soluble dithionites such as potassium dithionite or ammonium dithionite, a soluble bisulfite or metabisulfite such as sodium bisulfite, potassium bisulfite, lithium bisulfite, ammonium bisulfite, sodium metabisulfite, potassium metabisulfite, lithium metabisulfite or ammonium metabisulfite, or an aqueous solution of sulfur dioxide.

Technetium-99m in the form of an aqueous solution of sodium pertechnetate is readily obtainable from commercially available molybdenum-99/technetium-99m generators or alternatively, instant $^{99\text{m}}\text{Tc}$ may be used. Cu-64 is commercially available from Australian Nuclear Science & Technology Organisation and ^{67}Cu from the US Department of Energy, Brookhaven, USA.

The conjugate compounds of the eighth embodiment of this invention may be formed by the reaction of a radiolabelled metal complex of a compound of Formula (I) together with a molecular recognition unit. The radionuclides which are useful for complexing with the compounds of Formula (I) typically comprise metal ions which have at least two oxidation states, most typically an oxidation state of +2 or +3. In a typical embodiment, the radiolabelled metal complex is selected from ^{64}Cu , ^{67}Cu and $^{99\text{m}}\text{Tc}$, Sm, Ho, Re, Sc, Cu, Pd, Rh, and Y. The most typical metal ions comprise ^{64}Cu , ^{67}Cu , $^{99\text{m}}\text{Tc}$.

These radiolabelled metal complexes are then reacted with a molecular recognition unit. The radiolabelled molecular recognition unit so formed is useful for diagnostic, therapeutic and radioimaging applications.

Alternatively a conjugate of a compound of formula (I) may be first prepared, and then it may be radiolabelled.

Thus, radiolabelling of molecular recognition units such as proteinaceous materials using compounds of formula (I) can be conducted in two ways, namely:

- (a) prelabelling of a compound of Formula (I) with a suitable radionuclide, followed by conjugation of the resultant radiocomplexed compound to proteinaceous or other material,
- or
- (b) conjugating the compound of formula (I) to proteinaceous or other material for subsequent radiolabelling.

The formation of a conjugate compound of formula (I) is usually achieved by the reaction of the functionalised linkage group with a thiol, amino, carboxyl, hydroxyl, aldehyde, aromatic or heteroaromatic group present in the molecular recognition unit. For example, an amino or hydroxy group of the functionalised linkage group may be reacted with a free carboxyl group of the molecular recognition unit, or vice versa. Suitably a coupling agent such as a carbodiimide may be employed to facilitate the coupling reaction.

The conjugate compounds according to the eighth embodiment of the present invention may contain more than one molecule of a compound of formula (I) to any one molecular recognition unit. The metal complexing and radiolabelling of compounds of formula (I), and pharmaceutically acceptable salts thereof can be accomplished by using procedures recognised in the art. For example, the radiolabelling of the conjugate compounds with ^{64}Cu may be achieved by adding an aqueous acetate solution of ^{64}Cu to the conjugate compound in an aqueous solution and incubating for 5 minutes at room temperature. A composition comprising an uncomplexed conjugate in accordance with the invention may also be supplied to radio-chemists, technicians, radiopharmacists, doctors or the like in the form of a kit for radiolabelling immediately prior to use.

In a typical form of this invention, the kit comprises a first container that contains a radiolabelling metal ion, usually in solution, and a second container that contains a conjugate compound as described in the eighth embodiment of the present invention. The kit, in use, then involves mixing the contents of said first and second containers to obtain the radiolabelled conjugate compound.

Typically, the compounds of Formula (I) or the metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof are useful for labelling molecular recognition units for use in methods of diagnosis and therapy of disease. In particular, the typical radiolabelled molecular recognition units are monoclonal antibodies and fragments thereof, peptides, oligonucleotides, oligosaccharides or liposome or a part of a specific binding pair.

The applications of radiolabelled molecular recognition units comprise diagnosis, imaging and therapy of disease such as cancer. Typically, the compounds of formula (I) and their metal complexes and/or salts thereof have a diagnostic use as imaging agents *in vitro* and *in vivo*. The method of diagnosis using the aforesaid imaging agents will result from the localisation of the radiolabelled conjugate compounds on specific organs and tissues in a subject.

The method of diagnosis will typically involve first the administration of an effective amount of a radiolabelled compound of Formula (I) to a subject; and then monitoring the subject after a suitable period of time in order to ascertain the presence or absence of a cancer for example as evidenced by localisation of the radiolabel at a particular site in the subject. Typically, the monitoring step shall provide information regarding the location of any cancer if it is present. The effective amount or dosage of the radiolabelled compound of Formula (I) will depend upon the desired amount of radioactivity required for the diagnostic application balanced with the safety requirement of not exposing the subject, in particular their organs and tissues, to harmful amounts of radiation. Appropriate dosages for any given application may be determined by persons skilled in the relevant art by no more than routine experimentation, given the teaching herein.

The method of therapy will typically involve compounds of formula (I) or the metal complexes, radiolabelled complexes and/or pharmaceutically acceptable salts thereof which are useful as cytotoxic agents. In a typical embodiment, the therapy of disease comprises treatment of cancer, abnormal cell disorders and the treatment of tumours. In such applications, the radiolabelled compound of formula (I) is typically conjugated to a molecular recognition unit which is capable of binding specifically to the tumour or abnormal cell. Examples of such molecular recognition units comprise one part of specific binding pairs and are known to persons skilled in the relevant art and typically comprise antibody/antigen pairs and the like.

The method of therapy will typically involve the administration of an effective amount of a radiolabelled compound of Formula (I) to a subject. The effective amount or dosage will depend upon the desired amount of radioactivity required for the diagnostic application balanced with the safety requirement of not exposing the subject, in particular their organs and tissues, to harmful amounts of radiation. Appropriate dosages for any given application may be determined by persons skilled in the relevant art by no more than routine experimentation, given the teaching herein.

Typically the treatment would be for the duration of the condition, and contact times would typically be for the duration of the condition.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages of a compound of the present invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular vertebrate being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the compound of the present invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Also included within the scope of the present invention are prodrugs of the inventive compound. Typically, prodrugs will be functional derivatives of a compound of Formula (I) in accordance with the first embodiment of the invention, which are readily converted *in vivo* to the required compound for use in the present invention as described herein. Typical procedures for the selection and preparation of prodrugs are known to those of skill in the art and are described, for instance, in H. Bundgaard (Ed), *Design of Prodrugs*, Elsevier, 1985.

When used in the treatment of disease, the compound of Formula (I) in accordance with the first embodiment of the invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof, may be administered alone. However, it is generally preferable that these compounds be administered in conjunction with other chemotherapeutic treatments conventionally administered to patients for treating disease. For example, a tumour may be treated conventionally with surgery, and the compound of Formula (I) in accordance with the first embodiment of the invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof, to extend the dormancy of micrometastases and to stabilise and inhibit the growth of any residual primary tumour.

Typically, when used in the treatment of solid tumours, compounds of the present invention may be administered with chemotherapeutic agents such as: adriamycin, taxol, fluorouracil, melphalan, cisplatin, alpha interferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PROMACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, angiointibins, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide, SP-PG and the like. Other chemotherapeutic agents include alkylating agents such as nitrogen mustards including mechlorethamine, melphan, chlorambucil, cyclophosphamide and ifosfamide; nitrosoureas including carmustine, lomustine, semustine and streptozocin; alkyl sulfonates including busulfan; triazines including dacarbazine; ethylenimines including thiotepa and hexamethylmelamine; folic acid analogues including methotrexate; pyrimidine analogues including 5-fluorouracil, cytosine arabinoside; purine analogues including 6-mercaptopurine and 6-thioguanine; antitumour antibiotics including actinomycin D; the anthracyclines including doxorubicin, bleomycin, mitomycin C and methramycin; hormones and hormone antagonists including tamoxifen and corticosteroids and miscellaneous agents including cisplatin and brequinar.

When used in the treatment of disease, the compound of Formula (I) in accordance with the first embodiment of the invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof, may be administered alone. However, it is generally preferable that they be administered as pharmaceutical formulations. In general pharmaceutical formulations of the present invention may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

These formulations can be administered by standard routes. In general, the combinations may be administered by the topical, transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, oral, rectal or parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular) route. In addition, the compound of Formula (I) in accordance with the first embodiment of the invention or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof, may be incorporated into biodegradable polymers allowing for sustained release, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of for example, a tumour or implanted so that the active agents are slowly released systemically. Osmotic minipumps may also be used to provide controlled delivery of high concentrations of the active agents through cannulae to the site of interest, such as directly into for example, a metastatic growth or into the vascular supply to that tumour.

The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Examples of pharmaceutically and veterinarily acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower alkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butyleneglycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrrolidone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's
5 Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

The radiolabelled molecular recognition units especially radiolabelled antibodies are particularly useful in medicine, for example, in locating specific tissue types and in the therapy of cell disorders. The radiolabelled antibodies can also be used to target metal ions
10 to a specific tissue type, both *in vitro* and *in vivo*.

A typical use of the radiolabelled compounds of Formula (I) is to radiolabel monoclonal antibodies specific for colon, ovarian, lymphoma, breast and/or bladder cancer, with beta emitter radionuclides of metals such as Sm, Ho, Re, Sc, Cu, Pd, Pb, Rh and Y for therapy of above mentioned cancer. A further typical use is in the radiolabelling of a monoclonal antibody specific for metastasis of colon cancer for diagnosis and therapy.

In another typical embodiment, the antibody in the conjugate compound may be a complete antibody molecule or a fragment thereof or an analogue of either of these, provided that the antibody comprises a specific binding region. The antibody may be a humanised monoclonal or a fragment thereof. The antibody may also be a recombinant antibody. The antibody may be specific for any number of antigenic determinants, but is typically specific for one antigenic determinant.

In another typical embodiment, there is provided radiolabelling of monoclonal antibodies with ^{67}Cu (beta and gamma emitter) and ^{64}Cu (positron and beta emitter), for combined radioimmunoscinotography (RIS) (SPECT and PET) and radioimmunotherapy (RIT). Other radionuclides comprise Auger emitting agents where the compound of
25 Formula (I) is coupled to the monoclonal antibody and labelled with auger emitting isotope such as Fe-59 or Cu-64.

In still another typical embodiment, there is provided a two step pretargeted radioimmunotherapy where a monoclonal antibody with a first marker molecule attached thereto is injected into a subject. Once the antibody has cleared from the system and localised to the tumour, a second injection is administered to the subject. This second injection typically involves the radiolabelled complex of Formula (I) attached to a second marker molecule which recognises the first marker molecule on the targeted antibody. Alternatively, the second injection may typically be the second marker molecule alone and when cleared from the system, the radiolabelled complex of Formula (I) attached to the first
35 marker molecule is administered to the subject. Both procedures provide amplification of the target site and reduce exposure of the radiolabelled complex to normal tissue. Still typically, the first marker molecule is biotin and the second marker molecule is avidin or streptavidin. Still more typically, the first marker molecule is smaller than the targeted
40 antibody.

The invention also provides a two step procedure which involves the administration of an antibody-DNA conjugate or antibody-oligonucleotide conjugate followed by targeting with a radiolabelled complementary DNA or complementary oligonucleotide. This procedure also provides amplification of the target site and reduces exposure complex of the radiolabelled to normal tissue.

The invention also provides a use of compounds of Formula (I) or metal complexes, radiolabelled complexes, conjugate compounds or pharmaceutically acceptable salts thereof as Magnetic Resonance Imaging (MRI) agents. In this form of the invention, there is typically formed a complex of compound of formula (I) or a conjugate of the eighth embodiment, with a paramagnetic metal ion, typically Fe (III), Mn(II), which may be used as a contrast agent to enhance images. Further, complexes such as these may be employed in the form of a pharmaceutical formulation where the complex is present with a pharmaceutically acceptable carrier, excipient or vehicle therefor.

The pharmaceutical formulations described for the different embodiments of this invention typically comprise a formulation in the form of a suspension, solution or other suitable formulation. Physiologically acceptable suspending media together with or without adjuvants may be used. Still typically, the pharmaceutical formulations are in a liquid form and still more typically are in an injectable form. Still more typically, the injectable formulations are dissolved in suitable physiologically acceptable carriers which are recognised in the art.

The industrial use of the compounds of formula (I) further comprises their attachment to solid surfaces such as polymers, for use in the concentration of metal ions and purification of water or attached to an electrode surface for detection of specific metal ions.

Brief Description Of The Drawings

The present invention will be further illustrated, by way of example only, with reference to the accompanying figures; in which:

Figures 1a and 1b are graphical representations of the serum stability of ^{67}Cu complex of compound (4) at 0 and 7 days respectively;

Figure 2 is a graphical representation of the effect of pH on the complexation of ^{64}Cu with compound (3);

Figure 3 is a graphical representation of the biodistribution of a ^{64}Cu complex of compound (4);

Figure 4 is a graphical representation of the biodistribution of a ^{64}Cu complex of compound (1);

Figure 5 is a graphical representation of the biodistribution of a ^{64}Cu complex of compound (3); and

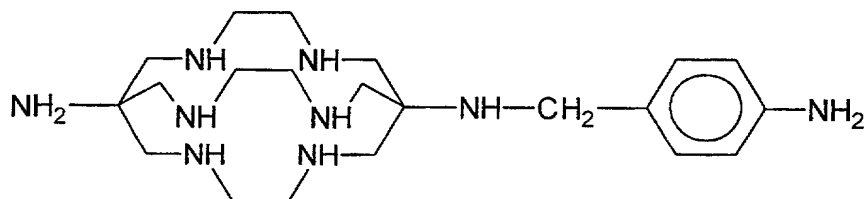
Figure 6 is a graphical representation of the biodistribution of ^{64}Cu labelled conjugate of B72.3 conjugated with compound (3) in Tumour Bearing Nude Mice.

Figure 7 is a graphical representation of the radiotherapeutic effect of 30MBq of ^{64}Cu labelled conjugate of B72.3 conjugated with compound (3) in Tumour Bearing Nude Mice.

Examples

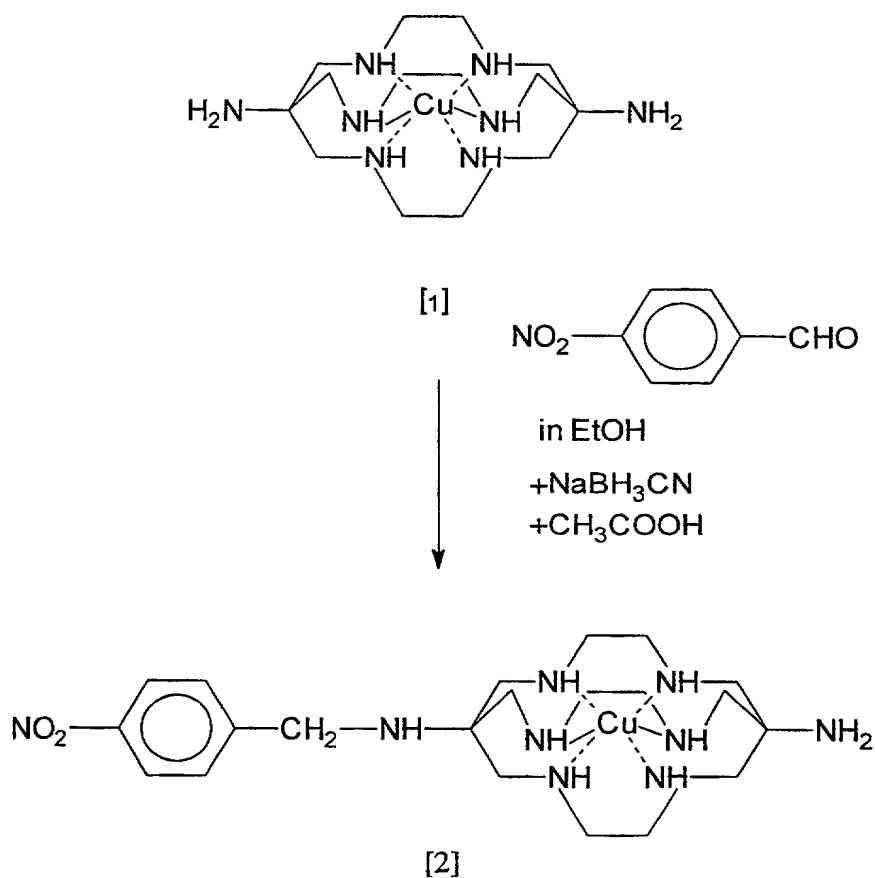
The following examples serve only to illustrate the invention and should not be construed as limiting the generality of the above description.

Example 1: Preparation of Compound (3)



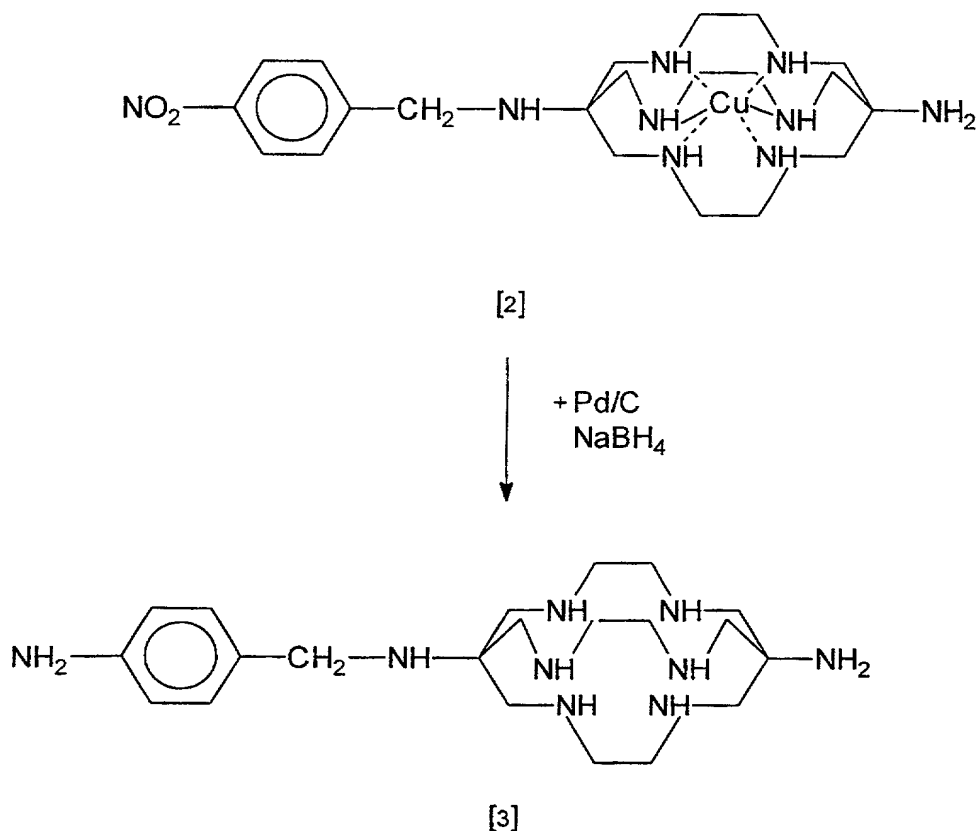
Compound (3)

Preparation of compound (3) is illustrated in Schemes 1 and 2.



17

Scheme 1. Schiff Base Condensation reaction of compound (1) with p-nitrobenzaldehyde



Scheme 2. Reduction of compound (2) to compound (3).

A. Preparation of Compound (2)

Compound (2) is prepared by Schiff base reaction of compound (1) with p-nitrobenzaldehyde. Synthesis of compound (1) is described in *Aust. J. Chem* (1994) **47**, 143–179. A copper complex of (1) is dissolved in dry ethanol (2.4 mmoles in 100 mls) and p-nitrobenzaldehyde (2.2 mmoles) is added. The reaction mixture is evaporated to dryness and reconstituted (with dry ethanol) twice to remove any water. The reaction mixture is once again reconstituted in dry ethanol and stirred for 30 mins under nitrogen gas. Sodium cyanoborohydride (25 mmoles), glacial acetic acid (2 mmoles) and activated 3 Å molecular sieves are added and the reaction is allowed to stir overnight. The mixture is filtered, evaporated to dryness and extracted with chloroform and water (100 ml : 200 ml). The water layer is diluted to 2 L, sorbed onto SP Sephadex C25 and eluted with 0.3 M sodium acetate. (Scheme 1).

B. Preparation of Compound (3)

To palladium/C catalyst (20 mg) in water (0.5 ml) is added sodium borohydride (50 mg) in water (0.5 ml) under nitrogen gas. To this mixture is then added compound (2) (30 mg) dissolved in approximately 0.1 M sodium hydroxide (0.5 ml). The mixture is left to stir at room temperature for a further 30 mins or until the solution becomes clear. A 2 ml glass vial is cooled on ice. The mixture is 0.22 μ m filtered into the cooled vial to remove the suspended palladium/C catalyst. To this cooled filtrate is added concentrated hydrochloric acid dropwise until all excess sodium borohydride is quenched (i.e. until gas evolution on addition of acid ceases). The quantity of product is determined by titration with a known concentration of ^{64}Cu as described in Example 2. Product is stored frozen at pH < 1 in a rubber-stoppered vial under nitrogen gas. Yield: > 95% (Scheme 2). The final product is characterised by ^1H -NMR in D_2O at 298K (Bruker Avance DPX 400). ^1H NMR 3.28 ppm, m, 6H, CH_2 (cage); 3.39 ppm, m, 6H, CH_2 (cage); 3.59 ppm, s, 6H, CH_2 (cage); 3.71 ppm, s, 6H, CH_2 (cage); 4.45 ppm, s, 2H, CH_2 ; 7.51 ppm, d, 2H, Ar; 7.70 ppm, d, 2H, Ar.

Example 2 Complexation of ^{64}Cu by Compound (3)

The effect of pH on complexation of ^{64}Cu by compound (3) was investigated. Compound (3) was diluted into buffers of pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. A sufficient amount of ^{64}Cu was added and the rate of complexation was monitored at (t = 1, 2.5, 5, 10, 60 and 90 mins) (see method below) by Instant Thin Layer Chromatography (ITLC-SG). Complete complexation (> 98 %) was achieved within 1 min for all pH \geq 4.0. The rate of complexation at pH 3.0 was slower. (Figure 2).

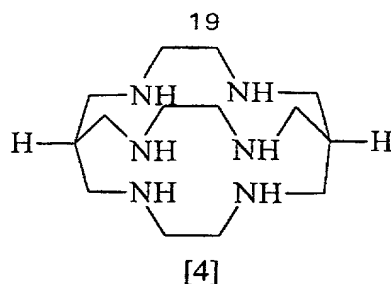
Monitoring Complexation by ITLC-SG

ITLC-SG strips (10 cm X 0.8 cm) were spotted with ~ 1 μ L of reaction mixture 1 cm from the bottom of the strip (origin) and were developed in a solvent containing 0.1 M sodium acetate (pH 4.5) : ethanol = 9:1. ^{64}Cu -compound (3) remains at the origin (R_f = 0.0), and "free" ^{64}Cu appears at the solvent front (R_f = 1.0).

A typical method of radiolabelling the ligand is achieved by adjusting the pH of an aqueous solution of the ligand to pH 5.0. Sufficient volume of $^{64/67}\text{Cu}$ solution (usually in 0.02 M HCl or diluted into 0.1 M sodium acetate buffer pH 5.0) is added to form a 1:1 complex. The efficacy of labelling is determined by ITLC-SG as described above. One main radiochemical species is observed.

Example 3 Serum Stability of ^{67}Cu complex of compound (4)

Serum stability studies were conducted by incubating a ^{67}Cu complex of related species (4) in human plasma at 37°C.



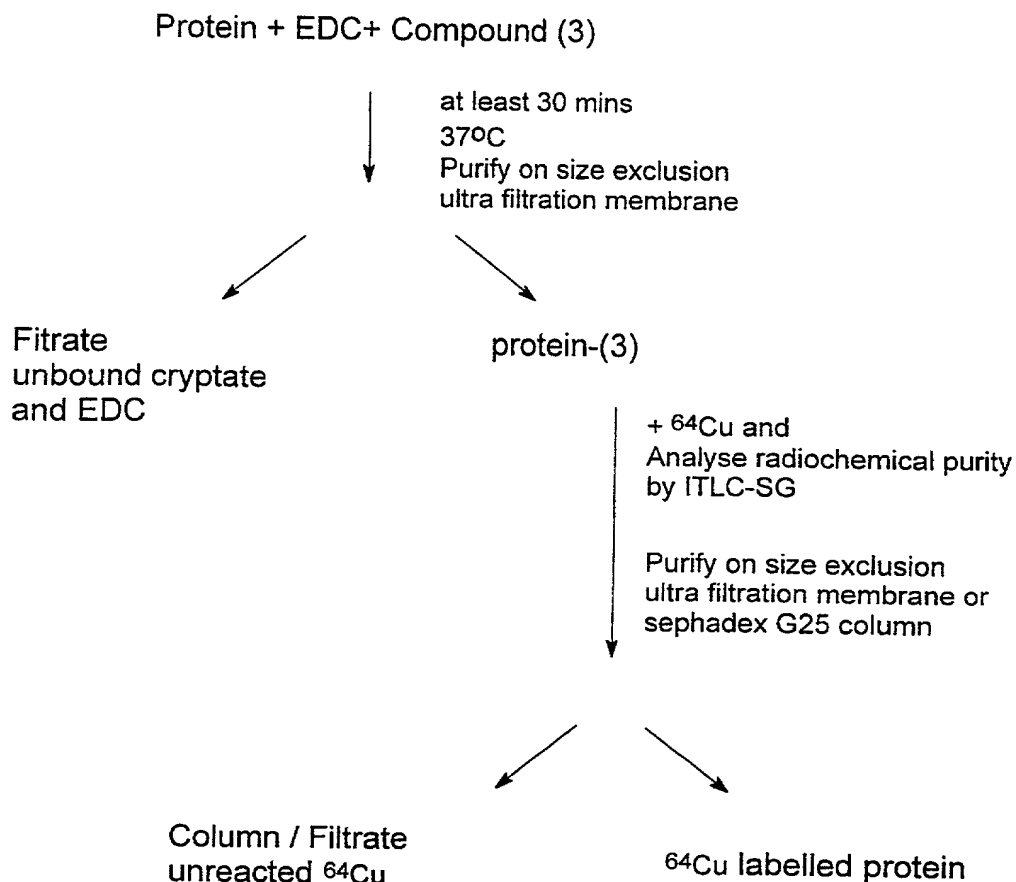
At various time intervals, the complex was separated from the plasma by size exclusion chromatography and the complex breakdown was assessed. Results indicated that no more than 2% of the copper is lost from the chelator during the first 174 hours at 37°C. (Figure 1a and 1b)

Example 4 Synthesis of B72.3 conjugated with (3) using 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC)

A typical method for radiolabelling an antibody (such as B72.3) is to incubate the antibody with the ligand (such as compound (3)) in the presence of EDC at pH 5.0 for 30 mins at 37°C. The unreacted ligand and EDC by-products are removed by washing with buffer (pH 5.0) on a size exclusion ultra filtration membrane. The purified immunoconjugate is exposed to a slight excess of $^{64/67}\text{Cu}$. The reaction is allowed to proceed at room temperature, and labelling is complete in less than 5 mins. Excess $^{64/67}\text{Cu}$ is removed by washing with 0.1 M EDTA in PBS (pH 7.2) on a size exclusion ultra filtration membrane or by separation on a size exclusion column (sephadex G25, eluted with PBS pH 7.2). (Scheme 3)

The conditions for radiolabelling B72.3 were optimised over incubation time (30 mins), reaction pH (5.0), concentration of B72.3 (5 mg/ml) and the molar ratio of B72.3:EDC:ligand (1:1000:250).

20



Scheme 3. Synthesis of protein conjugated with compound (3) using EDC.

Example 5 Biodistribution of Cu complexes of (1), (3) and (4) in Balb/C mice

The biodistribution of the ^{64}Cu complexes of compounds (1), (3) and (4) (0.10 ml intravenous injection) were evaluated in balb/c mice (5 animals per time point) at 3, 5, 10, 15, 20 and 30 minute time intervals. Biodistribution studies were performed in duplicate. Biodistribution of the radiolabel is presented in Tables 1, 2 and 3, and is illustrated diagrammatically in Figures 3, 4 and 5.

Example 6 Evaluation of ^{64}Cu -labelled conjugate of B72.3 with compound (3) in Tumour Bearing Nude mice

The B72.3 antibody recognises the TAG-72 antigen which is expressed on colorectal and ovarian tumours. The animal model used in the present study uses LS174t cells which also express TAG-72 antigen. The biodistribution of the ^{64}Cu immunoconjugate (0.10 ml intravenous injection) was evaluated in LS174t tumour bearing nude mice (5 animals per time point) at 1, 3, 5, 24 and 48 hour time intervals. Biodistribution studies were performed in duplicate. Biodistribution of the radiolabel is presented in Table 4, and is illustrated diagrammatically in Figure 6a; Biodistribution of complex of compounds (1) (2) and (3) show that if the ligand actually detaches from the antibody in any form it will clear rapidly from the system and not release the ^{64}Cu .

T07260" 22269860

TABLE 1.
⁶⁴Cu-labelled compound (4)

% INJECTED DOSE PER GRAM

TIME	3 MIN		5 MIN		10 MIN		15 MIN		20 MIN		30 MIN	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
ORGAN	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
LIVER	1.81	0.30	1.59	0.12	1.16	0.06	0.64	0.06	5.01	0.55	5.76	0.58
SPLEEN	2.26	0.43	2.17	0.29	1.28	0.14	0.67	0.15	0.52	0.08	0.35	0.07
KIDNEY	20.08	4.98	18.62	4.09	14.79	6.26	7.52	0.54	25.99	3.24	30.26	4.50
MUSCLE	1.76	0.23	2.10	0.59	1.24	0.27	1.73	1.32	0.36	0.06	1.27	1.43
SKIN	5.98	0.31	6.46	0.56	4.21	0.68	2.76	0.10	1.73	0.45	1.42	0.42
BONE	1.60	0.25	1.56	0.24	1.28	0.23	1.60	0.41	0.89	0.51	0.65	0.10
LUNGS	5.85	0.03	5.28	0.46	2.84	1.09	2.09	0.24	1.13	0.30	0.92	0.15
HEART	3.25	0.34	2.66	0.14	1.60	0.36	1.08	0.18	0.51	0.08	0.47	0.17
BLOOD	8.25	0.32	7.08	0.22	4.41	0.78	2.71	0.57	1.40	0.12	1.07	0.21
BLADDER	13.15	6.81	28.05	18.33	69.91	54.03	8.17	4.96	16.38	20.77	25.38	35.78
STOMACH	3.53	0.56	3.04	0.29	1.84	0.55	0.89	0.37	0.70	0.14	0.65	0.10
GIT	2.62	0.08	2.16	0.20	1.49	0.31	1.07	0.18	0.89	0.05	0.80	0.04

FOI 260 4269860

TABLE 2.
⁶⁴Cu-labelled compound (1)

% INJECTED DOSE PER GRAM		3 MIN		5 MIN		10 MIN		15 MIN		20 MIN		30 MIN	
TIME	ORGAN	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
	LIVER	2.29	0.22	1.50	0.17	1.42	0.22	0.82	0.19	0.69	0.15	0.50	0.08
	SPLEEN	2.09	0.52	1.48	0.21	1.54	0.25	1.44	0.76	0.69	0.12	0.52	0.16
	KIDNEY	23.18	11.73	11.50	1.34	12.80	1.83	6.77	1.40	7.77	0.67	4.95	0.92
	MUSCLE	1.81	0.32	1.81	0.59	2.39	1.14	2.28	1.66	1.17	1.02	1.33	1.59
	SKIN	5.82	0.42	5.59	0.59	5.48	1.07	2.61	0.44	2.96	0.72	1.63	0.08
	BONE	2.25	0.45	1.87	0.47	1.79	0.29	1.23	0.12	1.25	0.44	0.78	0.23
	LUNGS	6.51	1.28	4.45	0.54	3.91	1.98	2.06	0.32	2.73	0.61	0.85	0.31
	HEART	3.11	0.38	2.25	0.10	2.27	0.43	0.89	0.12	1.23	0.71	0.40	0.10
	BLOOD	8.68	1.35	6.32	0.70	5.45	0.81	2.48	0.31	2.63	0.29	1.14	0.25
	BLADDER	24.36	16.90	31.12	19.21	27.75	21.59	15.17	8.53	23.29	15.56	122.93	71.11
	STOMACH	3.50	0.64	2.73	0.36	2.08	0.80	1.50	0.46	1.16	0.06	0.74	0.04
	GIT	2.75	0.59	2.21	0.08	1.85	0.38	1.11	0.06	1.05	0.17	1.16	0.72

TABLE 3.
⁶⁴Cu-labelled compound (3)

TIME ORGAN	% INJECTED DOSE PER GRAM											
	3 MIN	5 MIN	10 MIN	15 MIN	20 MIN	30 MIN	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
LIVER	1.94	0.17	2.00	0.52	1.46	0.39	1.24	0.23	1.04	0.08	0.72	0.02
SPLEEN	1.96	0.14	2.01	0.16	1.26	0.20	1.09	0.25	0.93	0.13	0.39	0.05
KIDNEY	22.41	5.77	18.99	2.98	13.41	3.04	10.26	1.76	9.98	1.73	5.95	0.27
MUSCLE	1.98	0.48	3.17	2.34	1.73	0.28	2.40	1.39	2.13	1.35	0.50	0.01
SKIN	5.63	1.05	6.18	1.17	4.86	1.14	4.49	1.03	3.41	1.10	1.45	0.13
BONE	1.68	0.04	2.11	0.60	1.29	0.43	1.36	0.24	1.11	0.08	0.72	0.10
LUNGS	5.48	0.61	5.74	1.07	4.00	0.79	3.25	0.64	2.59	0.50	1.13	0.01
HEART	2.79	0.36	3.00	0.59	2.03	0.58	1.56	0.29	1.24	0.05	0.50	0.11
BLOOD	7.74	0.43	8.19	1.53	5.23	1.28	4.39	0.59	3.05	0.44	1.25	0.02
BLADDER	11.36	4.02	12.31	4.09	14.36	9.79	10.54	9.64	13.45	16.47	2.74	2.02
STOMACH	2.69	0.51	3.11	0.79	2.35	0.65	1.91	0.45	1.54	0.33	0.60	0.04
GIT	2.66	0.29	2.88	0.46	1.80	0.38	2.92	2.46	1.05	0.22	0.55	0.03

T01260" 22269860

TABLE 4.

⁶⁴Cu-labelled conjugate of B72.3 with compound (3)

% INJECTED DOSE PER GRAM

TIME ORGAN	1 HR		3 HR		5 HR		16 HR		24 HR		48 HR	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
LIVER	19.09	1.05	18.44	0.58	19.54	2.34	16.38	1.59	15.52	2.95	13.76	3.21
SPLEEN	11.51	1.13	8.35	1.19	10.17	0.98	10.08	1.75	8.47	1.89	8.44	1.57
KIDNEY	10.64	1.47	9.94	0.15	10.41	1.10	9.16	1.77	7.39	0.65	7.59	1.35
MUSCLE	1.45	0.52	1.48	0.23	1.45	0.16	1.98	0.27	2.54	0.54	1.72	0.42
SKIN	2.83	0.52	5.18	0.26	5.99	1.38	8.64	1.16	8.30	1.30	6.95	0.16
BONE	4.42	0.55	3.82	0.49	3.21	0.30	3.93	0.49	4.28	0.72	3.39	0.38
LUNGS	13.32	1.52	11.43	1.44	9.78	0.86	11.03	1.93	7.76	0.87	6.81	1.09
HEART	10.56	2.29	8.58	1.21	9.04	3.30	6.77	0.92	5.14	0.72	5.20	0.43
BLOOD	47.98	5.60	37.94	1.52	33.41	3.66	26.41	1.48	21.50	1.81	18.69	2.01
BLADDER	2.33	0.77	2.93	0.62	3.15	0.47	7.00	5.09	5.41	2.37	4.87	0.92
STOMACH	1.41	0.29	1.69	0.33	2.32	0.51	1.48	0.74	1.58	0.31	1.43	0.32
GIT	2.23	0.35	2.60	0.20	2.87	0.23	2.28	0.14	2.20	0.20	1.86	0.31
TAIL	5.03	0.73	4.96	0.83	5.64	1.82	6.14	2.47	5.18	1.20	4.08	0.30
TUMOUR	5.98	0.80	11.10	0.76	12.31	1.68	23.77	2.46	29.43	4.44	38.43	4.79

Table 5
Biodistribution of ^{125}I -B72.3 in nu/nu mice

% ID/g Time (hours) ORGAN	1		3		6		16		24		48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LIVER	11.89	2.33	8.93	1.24	7.45	1.51	5.61	0.64	5.03	0.82	4.92	0.90
SPLEEN	10.17	2.79	6.92	0.92	6.27	0.79	5.20	0.95	4.33	1.34	4.58	1.25
KIDNEY	11.20	0.77	8.89	1.40	9.24	0.53	5.83	0.88	5.40	0.98	4.76	0.78
MUSCLE	1.69	0.42	2.40	0.43	3.04	0.59	2.55	0.65	2.70	0.42	2.48	0.33
SKIN	4.36	1.85	5.96	0.94	7.79	0.76	7.94	0.95	7.96	0.78	7.41	1.44
BONE	5.43	0.54	4.43	0.69	3.23	0.43	3.19	0.78	2.58	0.58	2.54	0.36
LUNGS	14.28	2.97	12.84	2.89	11.00	1.57	7.39	1.62	6.64	1.57	6.47	0.64
HEART	14.03	6.26	10.11	1.16	9.46	1.59	7.28	2.50	5.78	0.62	4.87	1.10
BLOOD	55.63	3.36	44.78	4.03	42.20	3.93	28.27	3.00	28.73	2.23	22.73	2.50
URINE	3.96	0.58	13.29	5.00	18.31	6.92	7.28	4.17	6.92	1.11	6.87	2.11
BLADDER	3.49	1.15	5.10	1.63	4.85	0.91	4.74	1.24	5.70	1.13	6.63	1.23
STOMACH	1.80	0.23	2.94	0.44	4.06	0.50	2.64	0.90	3.06	0.99	3.05	0.72
GIT	2.35	0.26	2.74	0.29	2.74	0.39	1.84	0.33	1.65	0.26	1.51	0.07
THYROID	8.76	3.60	17.29	6.24	38.66	7.92	29.47	9.54	85.96	44.73	549.83	353.44
TUMOUR	9.61	2.04	16.46	1.61	31.43	8.39	31.37	6.54	44.67	8.50	46.17	7.25
%ID THYROID	0.08	0.02	0.19	0.03	0.42	0.11	0.40	0.18	1.24	0.64	2.59	1.13
TUMOUR:BLOOD	0.2	0.0	0.4	0.1	0.7	0.3	1.1	0.3	1.6	0.4	2.0	0.5
TUMOUR:LIVER	0.8	0.3	1.8	0.4	4.2	2.0	5.6	1.8	8.9	3.1	9.4	3.2
TUMOUR:KIDNEY	0.9	0.2	1.9	0.5	3.4	1.1	5.4	1.9	8.3	3.1	9.7	3.1
KIDNEY:BLOOD	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.1	0.2	0.0	0.2	0.1
KIDNEY:LIVER	0.9	0.2	1.0	0.3	1.2	0.3	1.0	0.3	1.1	0.4	1.0	0.3

Table 6.
Biodistribution of ¹¹¹In-DTPA-B72.3 in nu/nu mice.

% ID/g Time (hours) ORGAN	1		3		6		16		24		48	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LIVER	13.4	1.72	10.54	1.41	11.2	1.2	7.27	0.86	8.43	2.28	7.59	0.72
SPLEEN	12.52	2.66	9.56	1.96	10.05	2.55	6.47	3.33	7.32	1.43	4.48	2.28
KIDNEY	15.41	1.57	12.04	1.67	14.13	1.83	15.59	1.39	13.97	1.64	15.04	1.57
MUSCLE	2.08	0.81	1.96	0.42	2.56	0.32	2.44	0.82	2.28	0.26	1.84	0.51
SKIN	4.17	1.98	7.54	1.53	9.31	1.41	9.48	1.21	8.44	0.42	7.54	0.75
BONE	5.9	1.12	4.59	0.53	3.85	0.97	5.81	1.12	5.52	0.58	5.23	0.9
LUNGS	18.58	4.72	13.14	4.58	13.03	2.65	8.06	1.89	6.58	1.4	7.07	1.93
HEART	11.9	3.88	9.59	1.65	11.71	4.12	6.25	0.88	4.99	0.39	3.95	0.45
BLOOD	56.19	9.04	39.03	4.66	38.23	3.64	26.51	1.72	21.78	2.36	17.04	0.89
URINE			5.23	0	3.96	1.16	4.46	1.68	4.46	1.12	4.43	1.56
BLADDER	11.82	9.4	5.59	2.85	8.31	19.94	7.68	2.97	6.88	1.87	4.28	0.39
STOMACH	2.43	0.8	2.49	0.66	2.2	0.9	1.53	0.48	1.47	0.46	1.47	0.44
GIT	2.12	0.34	2.44	0.51	2.33	0.42	2.17	0.25	2.04	0.2	2.33	0.2
TAIL	9.37	5.68	8.39	4.38	8.53	5.27	4.98	1.17	4.69	0.96	3.79	1.37
THYROID	18.66	8.1	25.91	17.2	8.09	3.14	7.37	1.62	8.51	2.21	4.45	0.91
TUMOUR	8.87	2.39	17.95	4.93	21.24	6.11	38.25	8.71	35	5.21	48.99	4
%ID THYROID	0.07	0.01	0.06	0.03	0.07	0.01	0.07	0.02	0.06	0.03	0.06	0.01
TUMOUR:BLOOD	0.2	0.1	0.5	0.2	0.6	0.2	1.4	0.4	1.6	0.4	2.9	0.4
TUMOUR:LIVER	0.7	0.3	1.7	0.7	1.9	0.7	5.3	1.8	4.2	1.7	6.5	1.1
TUMOUR:KIDNEY	0.6	0.2	1.5	0.6	1.5	0.6	2.5	0.8	2.5	0.7	3.3	0.6
KIDNEY:BLOOD	0.3	0.1	0.3	0.1	0.4	0.1	0.6	0.1	0.6	0.1	0.9	0.1
KIDNEY:LIVER	1.2	0.3	1.1	0.3	1.3	0.3	2.1	0.4	1.7	0.6	2.0	0.4

It is to be understood that the term "SarAr" as used in the following tables and description refers to compound (3) as shown in example 1.

Table 7 Maximum Radiation Dose Estimates for ^{64}Cu -SarAr-B72.3

Organ	Total Dose mGy/MBq	Total Dose Rad/mCi
Adrenals	0.030	0.111
Brain	0.018	0.068
Breasts	0.021	0.078
Gallbladder Wall	0.036	0.133
LLI Wall	0.094	0.347
Small Intestine	0.047	0.175
Stomach	0.029	0.107
ULI Wall	0.069	0.255
Heart Wall	0.075	0.278
Kidneys	0.129	0.477
Liver	0.152	0.563
Lungs	0.062	0.230
Ovaries	0.026	0.095
Muscle	0.013	0.047
Pancreas	0.030	0.110
Red Marrow	0.029	0.107
Bone Surfaces	0.030	0.111
Skin	0.019	0.069
Spleen	0.088	0.327
Thymus	0.023	0.086
Testes	0.019	0.072
Thyroid	0.020	0.074
Bladder Wall	0.019	0.070
Uterus	0.029	0.109
Total Body	0.024	0.090

Tumour Mass (g)	S factor rad/uCi-h	Dose Gy	Dose rad
0.1	2.25	17.80	1779.75
0.5	0.48	3.80	380.47
1.0	0.25	1.97	196.96
2.0	0.13	1.00	99.67

Table 8 Maximum Radiation Dose Estimate for ^{131}I -B72.3

Organ	Total Dose mGy/MBq	Total Dose rad/mCi
Adrenals	0.821	3.038
Brain	0.514	1.902
Breasts	0.603	2.231
Gallbladder Wall	0.913	3.378
LLI Wall	2.24	8.288
Small Intestine	1.3	4.810
Stomach	1.15	4.255
ULI Wall	1.69	6.253
Heart Wall	1.82	6.734
Kidneys	2.41	8.917
Liver	1.92	7.104
Lungs	1.55	5.735
Muscle	0.473	1.750
Ovaries	0.768	2.842
Pancreas	0.858	3.175
Red Marrow	0.783	2.897
Bone Surfaces	0.678	2.509
Skin	0.547	2.024
Spleen	2.04	7.548
Testes	0.595	2.202
Thymus	0.717	2.653
Thyroid	44.6	165.020
Bladder Wall	0.755	2.794
Uterus	1.13	4.181
Total Body	0.715	2.646
Effective Dose	3.45 mSv/MBq	12.765 rem/mCi

(Tumour not included)

Tumour	S factor	Dose per mCi	
Mass (g)		Gy	rad
0.1	3.6	982	98275
0.5	0.759	207	20720
1	0.393	107	10728
2	0.2	54	5460

T07260" 2269860

Contribution to organ dose from activity in tumour

It is assumed that the tumour is a small source located in the lower trunk which will make a contribution to all other organ doses. So that the computer software program known as MIRDOSE3 can be used, the activity is assumed to be located in the ovaries.

- 5 The ovary dose given in above was calculated separately. It is not the tumour dose.

Bladder Residence Time - The activity excreted via the bladder is almost insignificant.

The total bladder and urine residence times in Table 5 was used as the urine activity in the dose calculation. No excretion model or assumed voiding time were used.

Table 9. Maximum Radiation Dose Estimates for ⁹⁰Y

Organ	Total Dose mGy/MBq	Total Dose rad/mCi
Adrenals	0.57	2.109
Brain	0.57	2.109
Breasts	0.57	2.109
Gallbladder Wall	0.57	2.109
LLI Wall	3.47	12.839
Small Intestine	1.36	5.032
Stomach	0.709	2.623
ULI Wall	2.25	8.325
Heart Wall	1.8	6.660
Kidneys	7.87	29.119
Liver	2.89	10.693
Lungs	1.88	6.956
Muscle	0.232	0.858
Ovaries	0.57	2.109
Pancreas	0.57	2.109
Red Marrow	0.964	3.567
Bone Surfaces	1.03	3.811
Skin	0.57	2.109
Spleen	2.32	8.584
Testes	0.57	2.109
Thymus	0.57	2.109
Thyroid	1.72	6.364
Bladder Wall	0.444	1.643
Uterus	0.57	2.109
Total Body	0.645	2.387

0986977.0910
TOT260.22269860

Tumour Mass (g)	S rad/uCi-h	Dose per mCi Gy	rad
0.1	8.97	546	54585
0.5	2.51	152.7	15274
1	1.4	85.2	8519
2	0.758	46.1	4613

Contribution to organ dose from activity in tumour

It is assumed that the tumour is a small source located in the lower trunk which will make a contribution to all other organ doses. So that the computer software program known as MIRDOSE3 can be used, the activity is assumed to be located in the ovaries. The ovary dose given in above was calculated separately. It is not the tumour dose.

Bladder Residence Time: The activity excreted via the bladder is almost insignificant.

The total bladder and urine residence times in Table 5 was used as the urine activity in the dose calculation. No excretion model or assumed voiding time were used.

Table 10. Radiation Dose Estimate for ^{131}I -B72.3 (7 days)

Organ	Total Dose mGy/MBq	Total Dose rad/mCi
Adrenals	0.377	1.395
Brain	0.24	0.888
Breasts	0.276	1.021
Gallbladder Wall	0.418	1.547
LLI Wall	1.02	3.774
Small Intestine	0.587	2.172
Stomach	0.52	1.924
ULI Wall	0.769	2.845
Heart Wall	0.863	3.193
Kidneys	1.13	4.181
Liver	0.888	3.286
Lungs	0.727	2.690
Muscle	0.212	0.784
Ovaries	0.35	1.295
Pancreas	0.393	1.454
Red Marrow	0.358	1.325
Bone Surfaces	0.309	1.143
Skin	0.25	0.925
Spleen	0.951	3.519
Testes	0.272	1.006
Thymus	0.328	1.214
Thyroid	17.8	65.860
Bladder Wall	0.338	1.251
Uterus	0.497	1.839
Total Body	0.323	1.195

Tumour	Dose to 24 hour
Mass (g)	rad/mCi
0.1	2991
0.5	631
1	327
2	166

Table 11. Radiation Dose Estimates for ⁹⁰Y (7 days)

Organ	Total Dose mGy/MBq	Total Dose rad/mCi
Adrenals	0.5	1.850
Brain	0.365	1.351
Breasts	0.5	1.850
Gallbladder Wall	0.5	1.850
LLI Wall	2.87	10.619
Small Intestine	1.14	4.218
Stomach	0.603	2.231
ULI Wall	1.87	6.919
Heart Wall	1.53	5.661
Kidneys	6.5	24.050
Liver	2.41	8.917
Lungs	1.58	5.846
Muscle	0.194	0.718
Ovaries	0.4	1.480
Pancreas	0.5	1.850
Red Marrow	0.808	2.990
Bone Surfaces	0.858	3.175
Skin	0.5	1.850
Spleen	1.97	7.289
Testes	0.5	1.850
Thymus	0.5	1.850
Thyroid	1.43	5.291
Bladder Wall	0.38	1.406
Uterus	0.5	1.850
Total Body	0.546	2.020

Tumour	rad/mCi at
Mass (g)	24 hour
0.1	6925
0.5	1938
1	1081
2	585

T07260" 44269850

Radiotherapeutic Study

Radiotherapeutic Study was conducted in two parts.

Part A where the theoretical doses to target and non-target organs were calculated for the analogous radioimmunoconjugates.

Part B where the various radioactive levels of ^{64}Cu -SarAr-B72.3 was injected into tumour bearing mice and the therapeutic effect of the product was monitored as an extension of animal survival time.

Radiotherapeutic Study - Part A

Biodistribution studies of ^{123}I - and ^{111}In - radiolabelled B72.3 were conducted in LS174t tumour bearing nude mice (see Table 5,6) Standard calculations were performed using computer software MIRDOSE 3 which was used to compare target to non-target dose of their analogous therapeutic counterparts (^{90}Y and ^{131}I respectively) with ^{64}Cu -SarAr-B72.3 (see Table 7,8,9)

Theoretical maximum accumulated dose (which is equivalent to 10 half life decay) for each radioimmunoconjugate was calculated. Total body dose for ^{64}Cu -SarAr-B72.3 was significantly lower (0.09 rad/mCi) than analogous products (^{131}I -B72.3, 2.64 rad/mCi; ^{90}Y -B72.3, 2.387 rad/mCi). Comparative maximum doses to tumours of various sizes was calculated. Doses for ^{131}I - and ^{90}Y -B72.3 appear to be better than for ^{64}Cu -SarAr-B72.3. However, the lack of stability of the radioimmunoconjugate at the tumour site in real biological systems prevents the tumour from receiving the maximum accumulated dose.

Hence doses to target and non-target organs were re-calculated assuming the radioimmunoconjugates (^{131}I and ^{90}Y) were stable for approximately 24 hours at the tumour site and the non-target organs were dosed for up to 7 days allowing for natural biological clearance. MIRDOSE 3 was used to re-calculate dose to various organs under these conditions (see Table 10,11). Most relevant resultant target to non-target ratios are given in Table 12.

Table 12 Ratio of Target:Non-Target Doses for each Radioimmunoconjugate

	^{64}Cu	^{131}I	^{90}Y
Tumour (0.1g):Kidneys	2094	716	288
Tumour (0.1g):Liver	1795	906	776
Total Body Dose for 7 days	0.09	1.195	2.020

*MIRDOSE 3 was used to estimate human organ doses assuming the residence times in man are the same as the animal model. It is acknowledged that this affects the accuracy of the dose estimates.

Radiotherapeutic Study - Part B.

In order to assess therapeutic effect of ^{64}Cu , in a real biological system, nude mice bearing LS174t colorectal carcinoma were injected with various doses (0, 10, 20, 30, 40 MBq) of ^{64}Cu -SarAr-B72.3.

Results are given in Table 13 and a typical profile of a study is given in Figure 1. For all activity levels greater than 20 MBq a significant extension in mouse life was achieved. Experimental details follow.

Table 13 Extension of mouse life relative to ^{64}Cu -SarAr-B72.3 - Part B

Experiment	Survival ¹ (Days)
Control ²	20-25
10 MBq	30-35
20 MBq	60-70
30 MBq	40-45
40 MBq	30-35

¹Greater than 30 % of animals; ²Greater than 50 control animals;

Figure 1. Radiotherapeutic effect of 30 MBq of ^{64}Cu -SarAr-B72.3 in tumour bearing mice.

Experimental for Radiotherapeutic Study - Part B

Animal model: LS174t tumour tissue from nude mice was transplanted into nude mice for each experiment.

Animal selection: Only animals bearing tumours 3.5 - 5.5 mm

7 days post transplantation were selected for each study. Up to 10 animals per dose.

Injection: The product was injected into the nude mice on Day 7 after transplantation of tumour tissue.

Doses: Various activities of Cu-64-SarAr-B72.3 was injected into the mice. Control animals receive only antibody.

Monitoring of animals: Animals were monitored for changes in tumour size

Animal mass

Behavioural and physical abnormalities

(e.g. movement / gait , food intake and hunching)

(For any of the above characteristic, frequency of weighing increased)

Histology, Haematology and Biochemistry:

Animals were sacrificed at pre-determined time points (2 days, 1, 2, 3, 4 weeks, 2, 3, 4, 5 and 6 months).

Radiotoxic effects were monitored.

(conducted by external pathologist of Department of Veterinary Anatomy and Pathology, University of Sydney)

Endpoint of the study:

1) Body weight loss > 20 %.

- 2) Rapid weight loss of > 10% overnight.
- 3) Ulceration of tumour
- 4) Limitation of normal behaviour (e.g. ability to feed or drink).
- 5) Tumour size 10 x 10 mm (UK Cancer Council)

5 **Example 7**

In accordance with the description of the invention provided above specific preferred pharmaceutical compositions of the present invention may be prepared, and examples of which are provided below. The following specific formulations are to be construed as merely illustrative examples of formulations and not as a limitation of the scope of the present invention in any way.

A compound of Formula (I) may be administered alone, although it is preferable that it be administered as a pharmaceutical formulation.

Example 7(a) - Composition for Parenteral Administration

A pharmaceutical composition of the present invention for intramuscular injection could be prepared to contain 1 mL sterile isotonic saline, and 1 mg of compound of Formula (I).

Similarly, a pharmaceutical composition for intravenous infusion may comprise 250 ml of sterile Ringer's solution, and 5 mg of compound of Formula (I).

Example 7(b) - Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection may be prepared by mixing 1% by weight of compound of Formula (I) in 12% by volume propylene glycol and isotonic saline. The solution is sterilised by filtration.

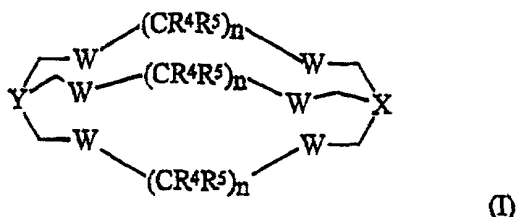
Modifications and variations such as would be apparent to a skilled addressee are deemed to be within the scope of the present invention. It is to be understood that the present invention should not be limited to the particular embodiment(s) described above. Throughout the specification, unless the context clearly indicates otherwise, the word, "comprise", "comprises", "comprising" or other variations thereof shall be understood as meaning that the stated integer is included and does not exclude other integers from being present even though those other integers are not explicitly stated.

Further, the present invention relates to all steps, compounds, intermediates as well as final products.

AMENDED CLAIMS

[received by the International Bureau on 08 May 2000 (08.05.00);
original claims 1-24 replaced by new claims 1-23 (5 pages)]

1. A compound which is capable of being radiolabelled of general formula (I) which is as follows:

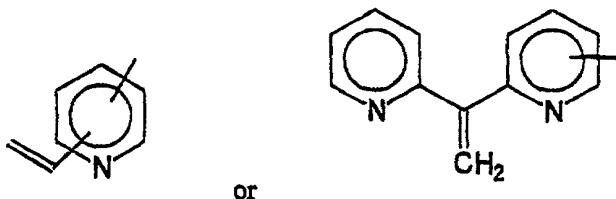


- 5 in which n represents an integer from 2 to 4,
where each R⁴ and R⁵ is independently selected from -H, CH₃, COOH, NO₂, CH₂OH, H₂PO₄, HSO₃, CN, C(=O)NH₂ and CHO;

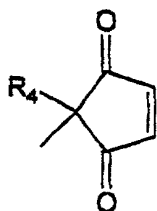
- X and Y are the same or different and are selected from the group consisting of C-R, N, P and C-Z in which R is selected from hydrogen, halogen, hydroxyl, nitro, nitroso,
10 amino, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, cyano, COOR', COCOOR', NH-COCH₂Br and -NH-CO-CH=CH-COOR' in which R' is a hydrogen atom or alkyl group;

W is selected from the group consisting of NH, S and O; and

- Z is a functionalised linkage group which is capable of binding said compound of
15 formula (I) to a molecular recognition unit, selected from the group consisting of C(=NH)OR², NCO, NCS, SR², NHNHR²R³, NHCONHNHR²R³, NHCSNHNHR²R³, CONR²R³, NR²R³, (CH₂)_pR⁶, (CH₂)_pArR¹, (CH₂O)_pCH₂R¹, (CH₂OCH₂O)_qArR¹, (CHCH)_rR¹, (CHCH)_rArR¹, maleimide, a vinyl pyridyl group of formula



- 20 a dione of formula



and a substituted vinyl group of formula $\text{Het}^1\text{-C}(\text{Het}^2)=\text{CH}_2$, where Het^1 and Het^2 are the same or different and each is a nitrogen containing heterocyclic group or Het^1 is a nitrogen containing heterocyclic group and Het^2 is H; where

R^2 and R^3 are the same or different and are independently selected from H, $(\text{CH}_2)_p\text{R}^1$, $(\text{CH}_2)_p\text{ArR}^1$, $(\text{CH}_2\text{O})_p\text{CH}_2\text{R}^1$, $-(\text{CH}_2\text{OCH}_2\text{O})_q\text{ArR}^1$, $(\text{CHCH})_r\text{R}^1$ and $(\text{CHCH})_r\text{ArR}^1$, with the proviso that when R^2 is H, R^3 is not H;

R^1 is selected from SH, OH, NH_2 , COOH, NCS, $-\text{N}=\text{N}$, or $-\text{C}(=\text{NH})\text{OCH}_3$ and, COR'' , where R'' is H, halogen, N_3 , alkoxy, OAr, imidyloxy, imidazoyloxy, alkyl, or alkyl substituted with a halogen or other leaving group; and

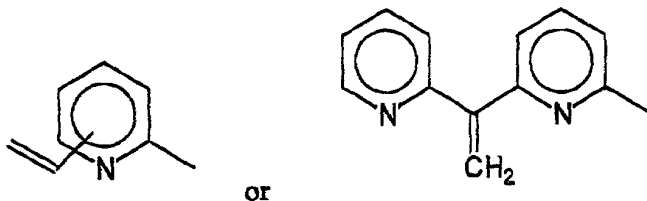
R^6 is selected from SH, NH_2 , COOH, NCS, $-\text{N}=\text{N}$, or $-\text{C}(=\text{NH})\text{OCH}_3$ and, COR'' ; and

p is an integer from 1 to 20, q is an integer from 1 to 20, r is an integer from 1 to 4, and Ar is optionally substituted aryl or optionally substituted aralkyl; and wherein

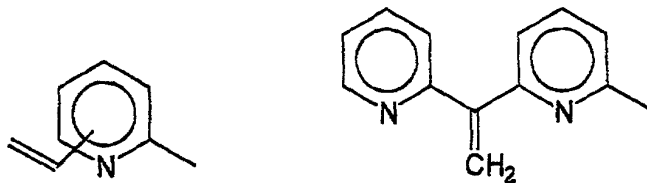
at least one of X and Y is C-Z, or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 wherein the molecular recognition unit is selected from the group consisting of an antibody, protein, peptide, carbohydrate, nucleic acid, oligonucleotide, oligosaccharide and liposome.

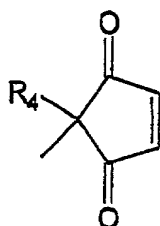
3. A compound according to claim 1, wherein the functionalised linkage group Z of the compound of Formula (I) is a vinyl pyridyl group of formula



4. A compound according to claim 1, wherein the functionalised linkage group Z of the compound of Formula (I) is selected from



a dione of formula



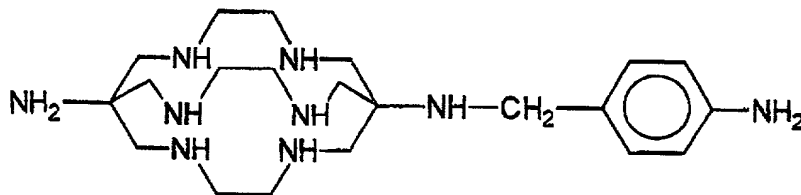
, and

NR²R³ where R² and R³ are the same or different and are independently selected from H, (CH₂)_pR¹, (CH₂)_pArR¹, (CH₂O)_pCH₂R¹, -(CH₂OCH₂O)_qArR¹, -(CHCH)_rR¹, and (CHCH)_rArR¹, with the proviso that when R² is H, R³ is not H, and where R¹ is selected from NH₂, COOH, NCS, NCO, -N=N-, -C(=NH)OCH₃, and COR'' where R'' is H, halogen, alkyl, or alkyl substituted with a halogen or other leaving group, where p is an integer from 1 to 20; q is an integer from 1 to 20; r is an integer from 1 to 4; and Ar is optionally substituted aryl or optionally substituted aralkyl, provided that at least one of R² and R³ is other than hydrogen.

5. A compound according to claim 1, wherein W is NH and Z is selected from NR²R³ where R² and R³ are the same or different and are independently selected from H, (CH₂)_pR¹, and (CH₂)_pArR¹, with the proviso that when R² is H, R³ is not H; R¹ is selected from NH₂, COOH and NCS; and p is an integer from 1 to 4.

6. A compound according to claim 4, wherein the Z group of said compound of Formula (I) is NR²R³ where R² and R³ together with the nitrogen atom to which they are attached form an optionally substituted saturated or partially unsaturated ring optionally containing one or more further heteroatoms O, S or N whereby there is at least one substituent capable of binding said compound of Formula (I) with a molecular recognition unit.

7. A compound of Formula (I) having the following structure:



8. A compound according to claim 1, wherein said compound is complexed with a metal ion.

9. A compound according to claim 8 wherein the metal ion is selected from Cu, Tc, Gd, Ga, In, Y, Co, Re, Fe, Au, Ag, Rh, Pt, Bi, Cr, W, Ni, V, Pb, Ir, Pt, Zn, Cd, Mn, Ru, Pd, Hg, Ti, and the lanthanide group of elements in the Periodic Table such as Sm, Ho, Gd, Tb, Sc.

10. A compound according to claim 9 wherein the metal ion is a radionuclide selected from the group of Cu, Cu, Tc, In, Gd, Ga, Fe, Cu, Ti and other radionuclides from the Lanthanides, Re, Sm, Ho, and Y.

11. A compound according to claim 10 wherein the radionuclide is selected
5 from ^{64}Cu and ^{67}Cu .

12. A pharmaceutical formulation comprising a compound according to claim 1, a radiolabelled complex or pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

13. A diagnostic formulation comprising a compound according to claim 1, a
10 radiolabelled complex or pharmaceutically acceptable salt thereof and a reducing agent in a pharmaceutically acceptable carrier.

14. A method of diagnosis or therapy in a subject comprising administering to the subject a diagnostically or therapeutically effective amount of a compound of Formula (I) according to claim 1 or a metal complex, radiolabelled complex or a pharmaceutically
15 acceptable salt thereof.

15. Use of a compound according to claim 1 or a metal complex, radiolabelled complex or pharmaceutically acceptable salt thereof in the preparation of a medicament for diagnosis or therapy of disease in a subject.

16. A compound according to claim 1 or a metal complex, radiolabelled
20 complex or pharmaceutically acceptable salt thereof when used in the diagnosis or therapy of disease in a subject.

17. A conjugate compound comprising at least one compound of Formula (I) according to claim 1 or a metal complex, radiolabelled complex or a pharmaceutically acceptable salt thereof bonded to at least one molecular recognition unit comprising an
25 antibody, protein, peptide, carbohydrate, oligonucleotide, oligosaccharide.

18. A method of diagnosis or therapy in a subject comprising administering to the subject a diagnostically or therapeutically effective amount of a conjugate compound according to claim 17.

19. Use of a conjugate compound according to claim 17 in the preparation of a
30 medicament for diagnosis or therapy of disease in a subject.

20. A conjugate compound as described in claim 1 when used in the diagnosis or therapy of disease in a subject.

21. A method of imaging a subject comprising introducing a compound of Formula (I) according to claim 1 or a metal complex, radiolabelled complex, conjugate
35 compound or pharmaceutically acceptable salt thereof to a subject.

22. Use of a compound of Formula (I) according to claim 1 or a metal complex, radiolabelled complex, conjugate compound or pharmaceutically acceptable salt thereof in the preparation of a medicament for imaging in a subject.

23. A compound of Formula (I) according to claim 1 or a metal complex,
5 radiolabelled complex, conjugate compound or pharmaceutically acceptable salt thereof when used in imaging in a subject.

TOT260 2269860

5. JUL. 2001 17:59

SPRUSON & FERGUSON 61 2 92615486

09/869777
NO. 3961 P. 49

WO 00/40585

PCT/AU00/00003

1/8

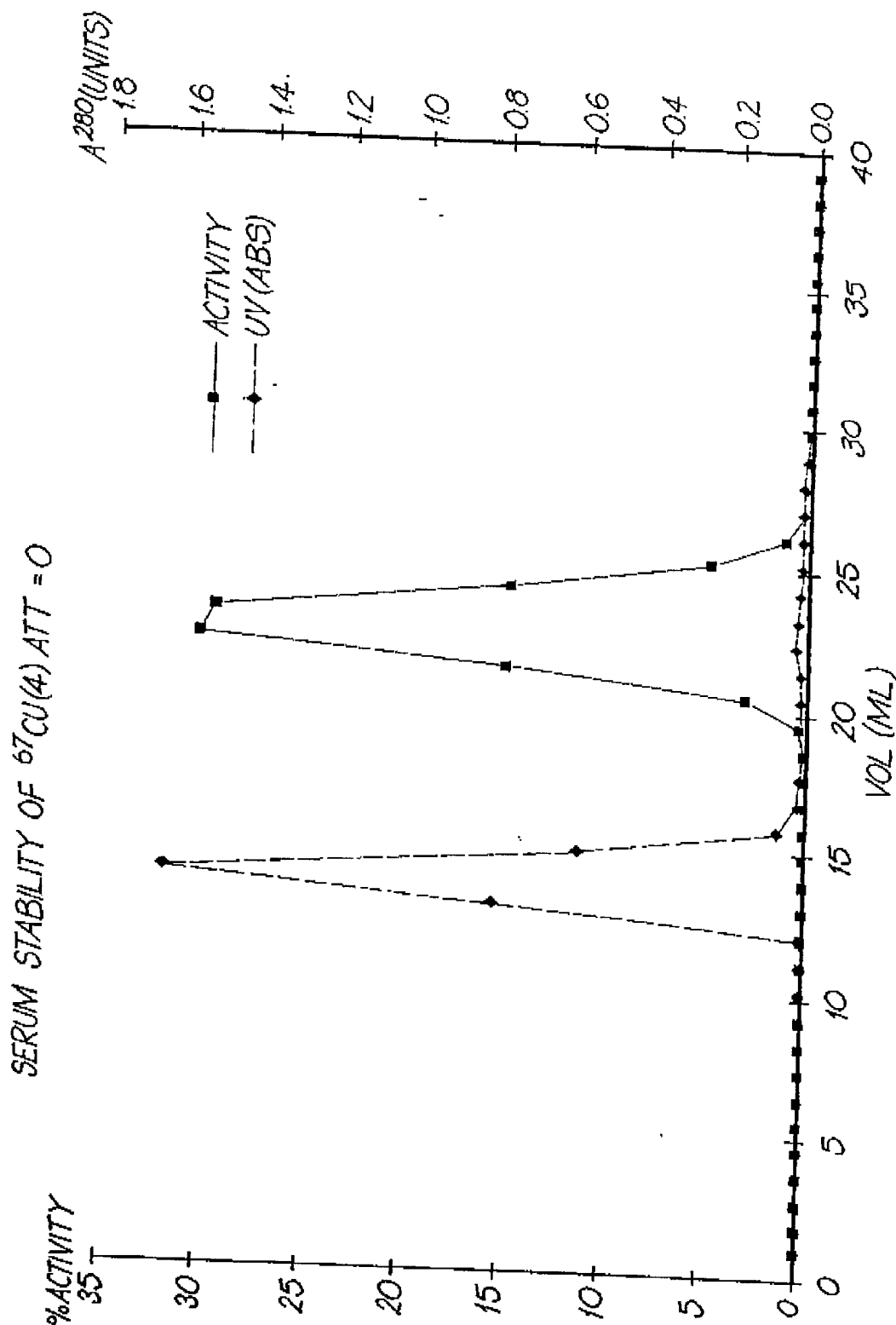


FIG. 1a

TOPSECRET

5. JUL. 2001 17:59

SPRUSON & FERGUSON 61 2 92615486

09/869777
NO. 3961 P. 50

WO 00/40585

PCT/AU00/00003

2/8

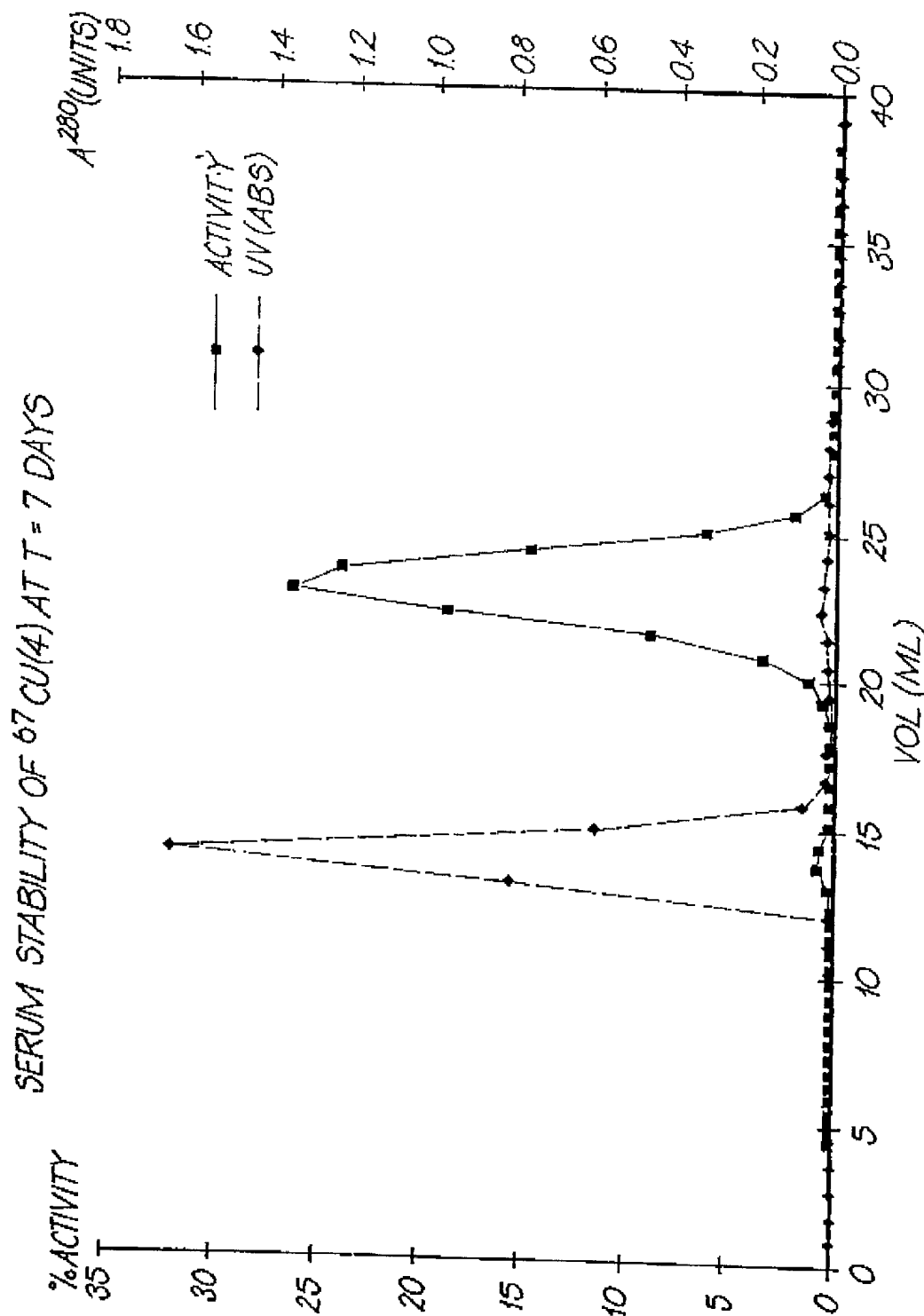
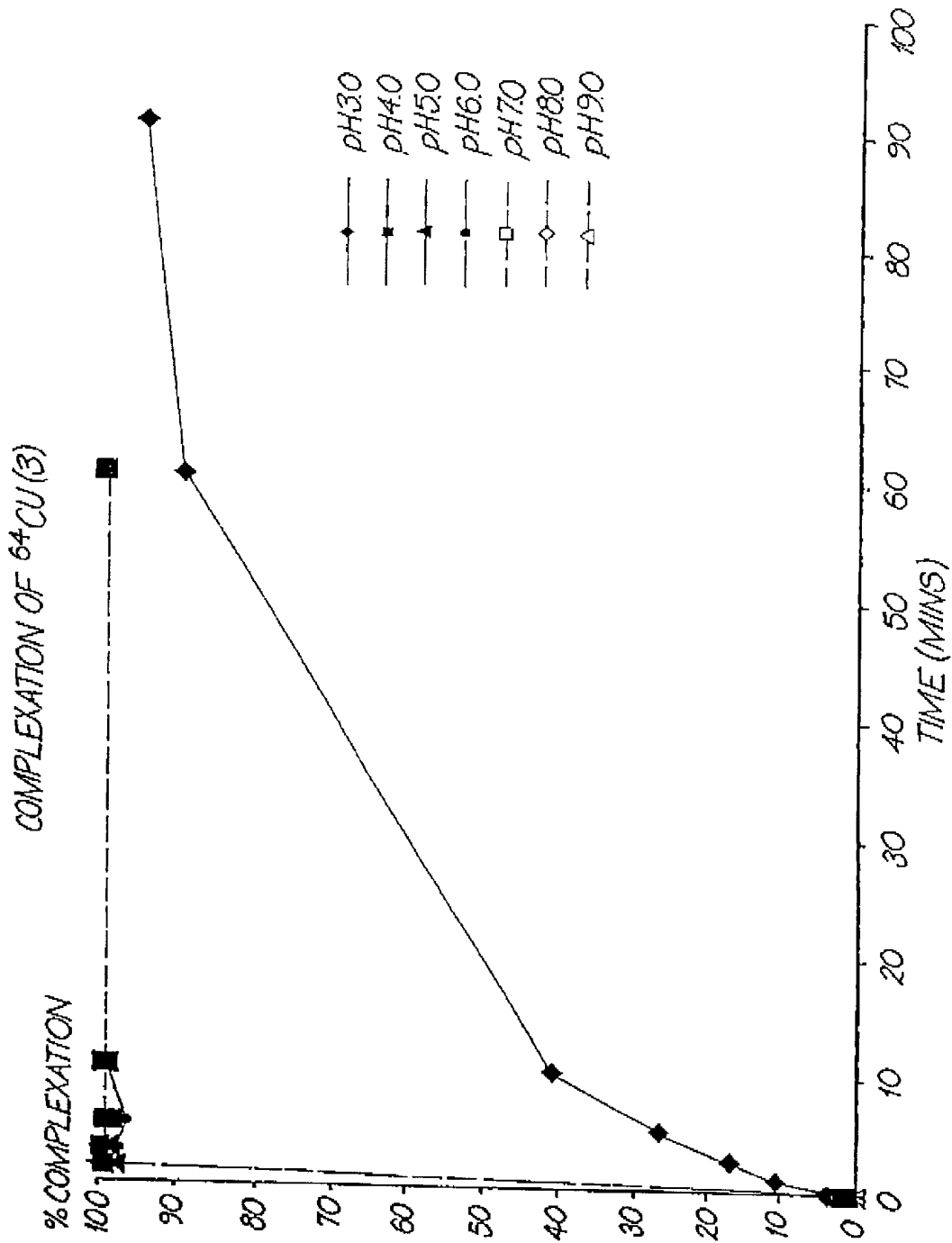


FIG. 1b



WO 00/40585

PCT/AU00/00003

4/8

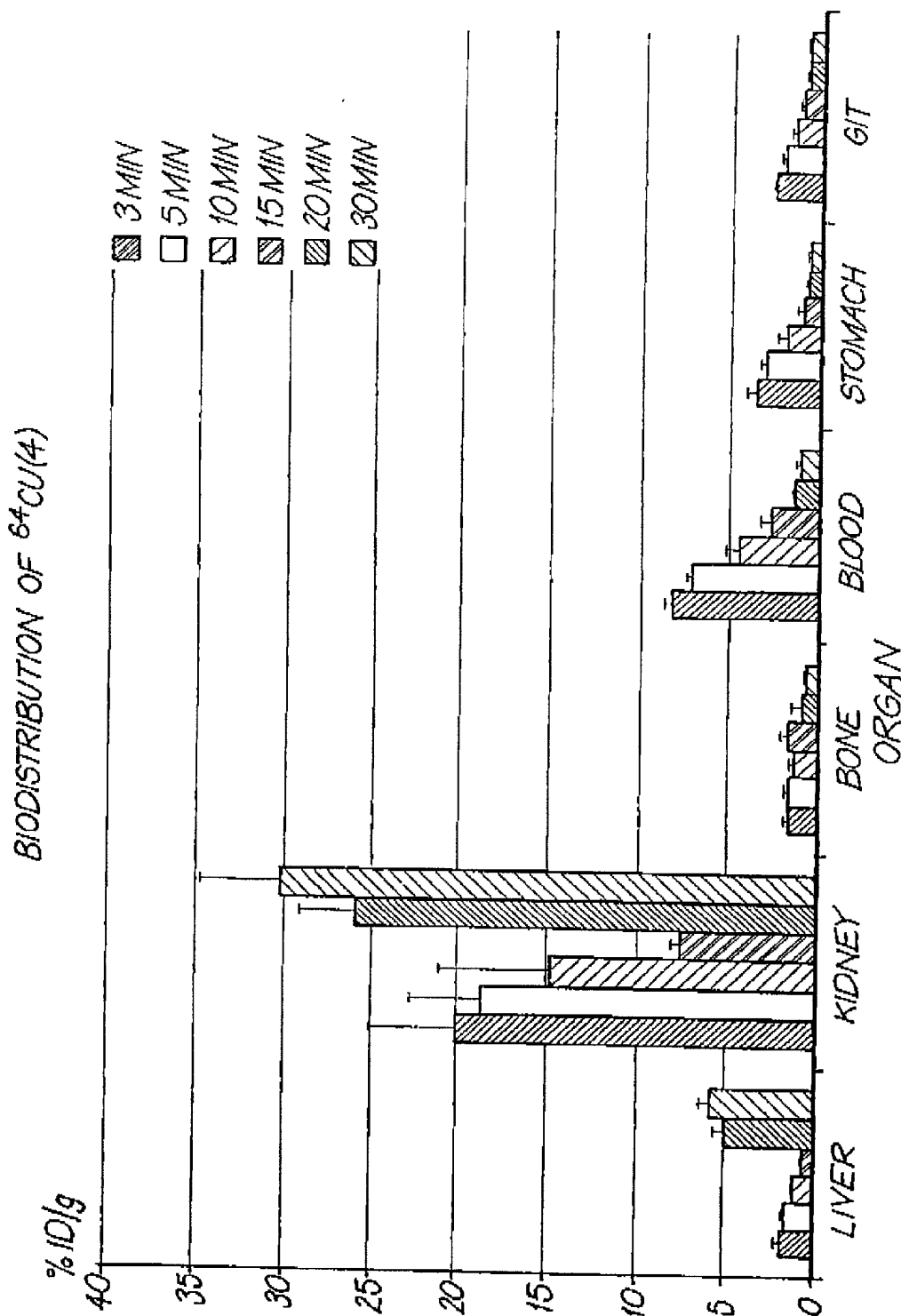


FIG. 3

TOP SECRET

WO 00/40585

PCT/AU00/00003

5/8

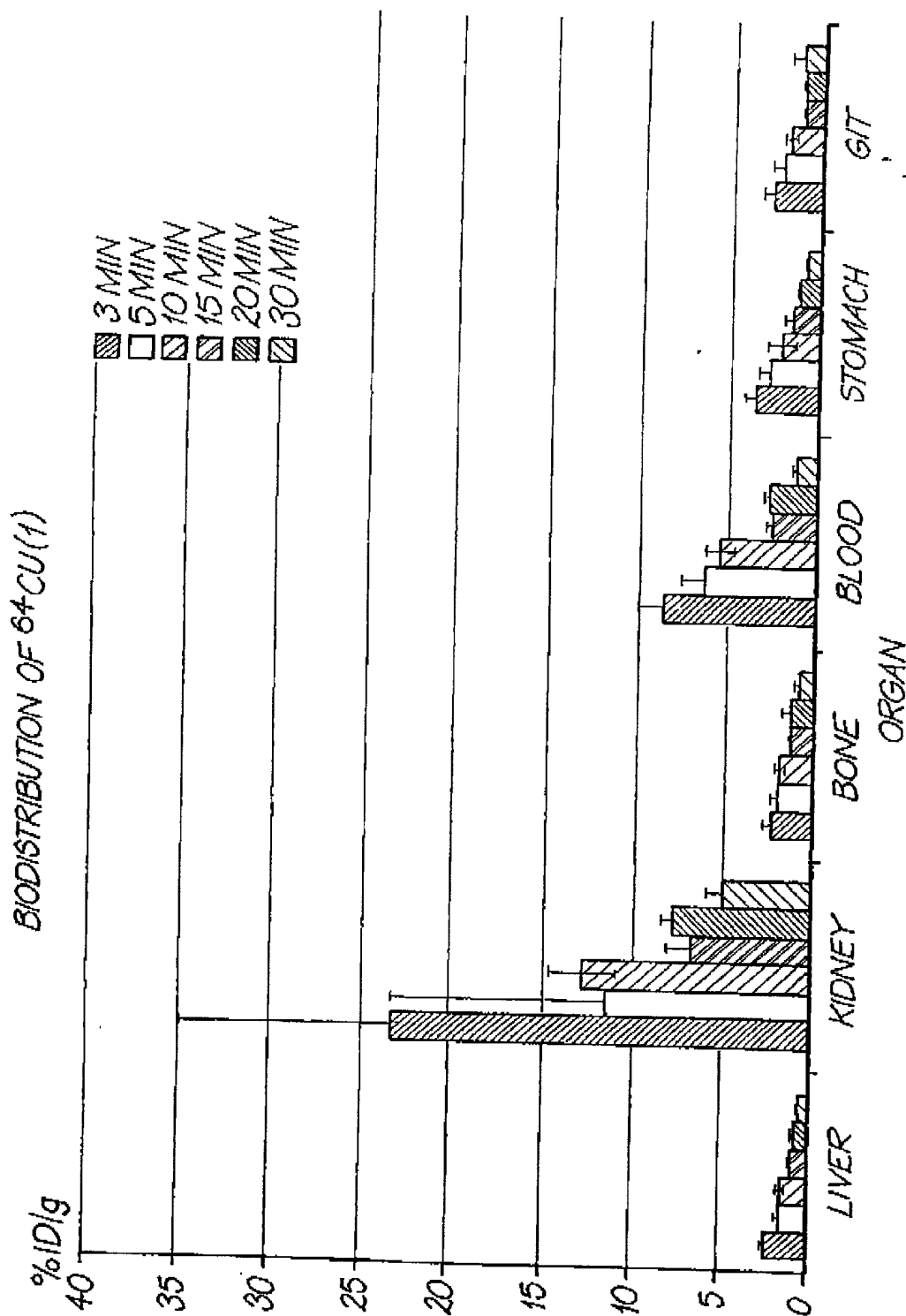


FIG. 4

TOT260 22269860

WO 00/40585

PCT/AU00/00003

6/8

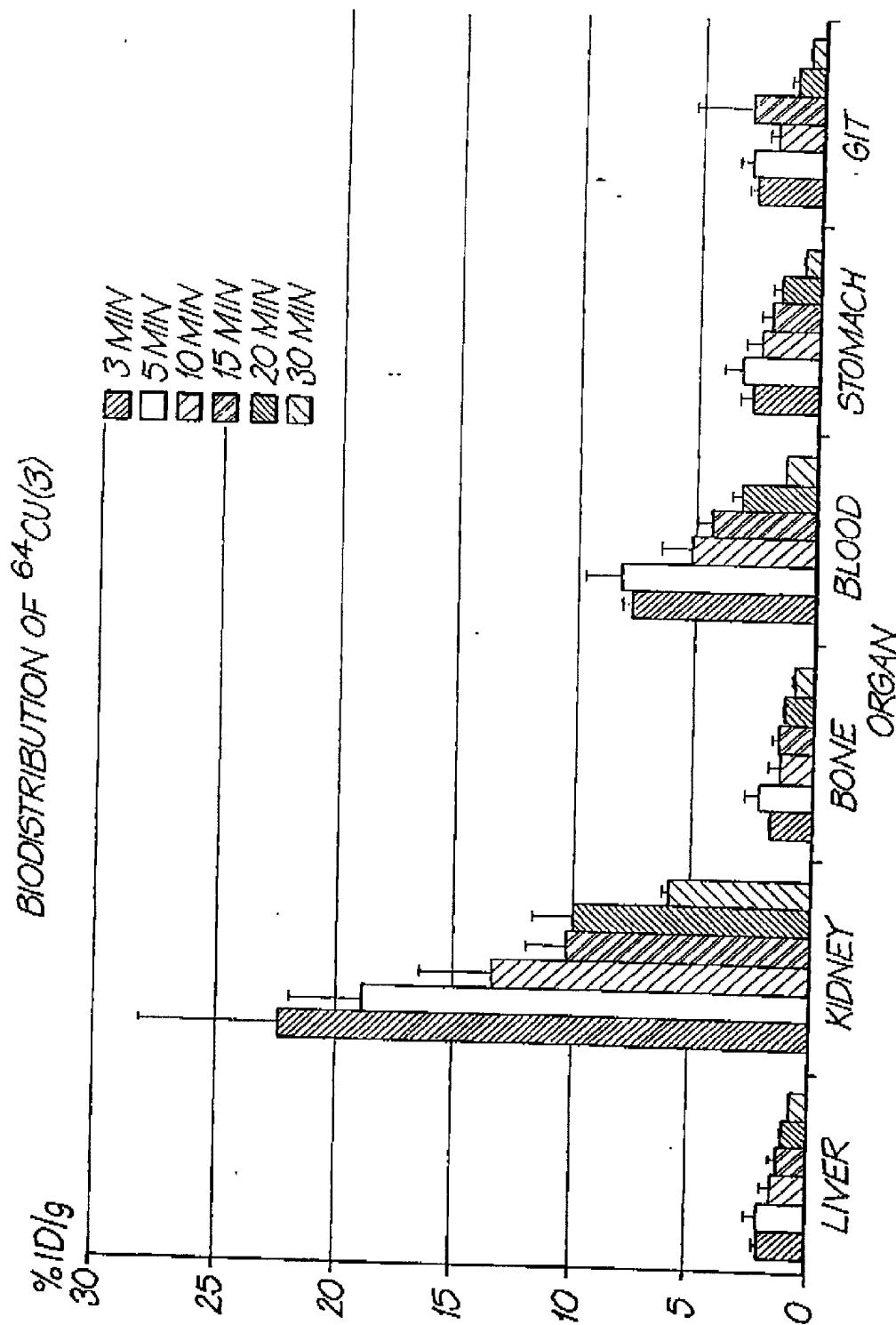


FIG. 5

T01260" 44459860

WO 00/40585

PCT/AU00/00003

7/8

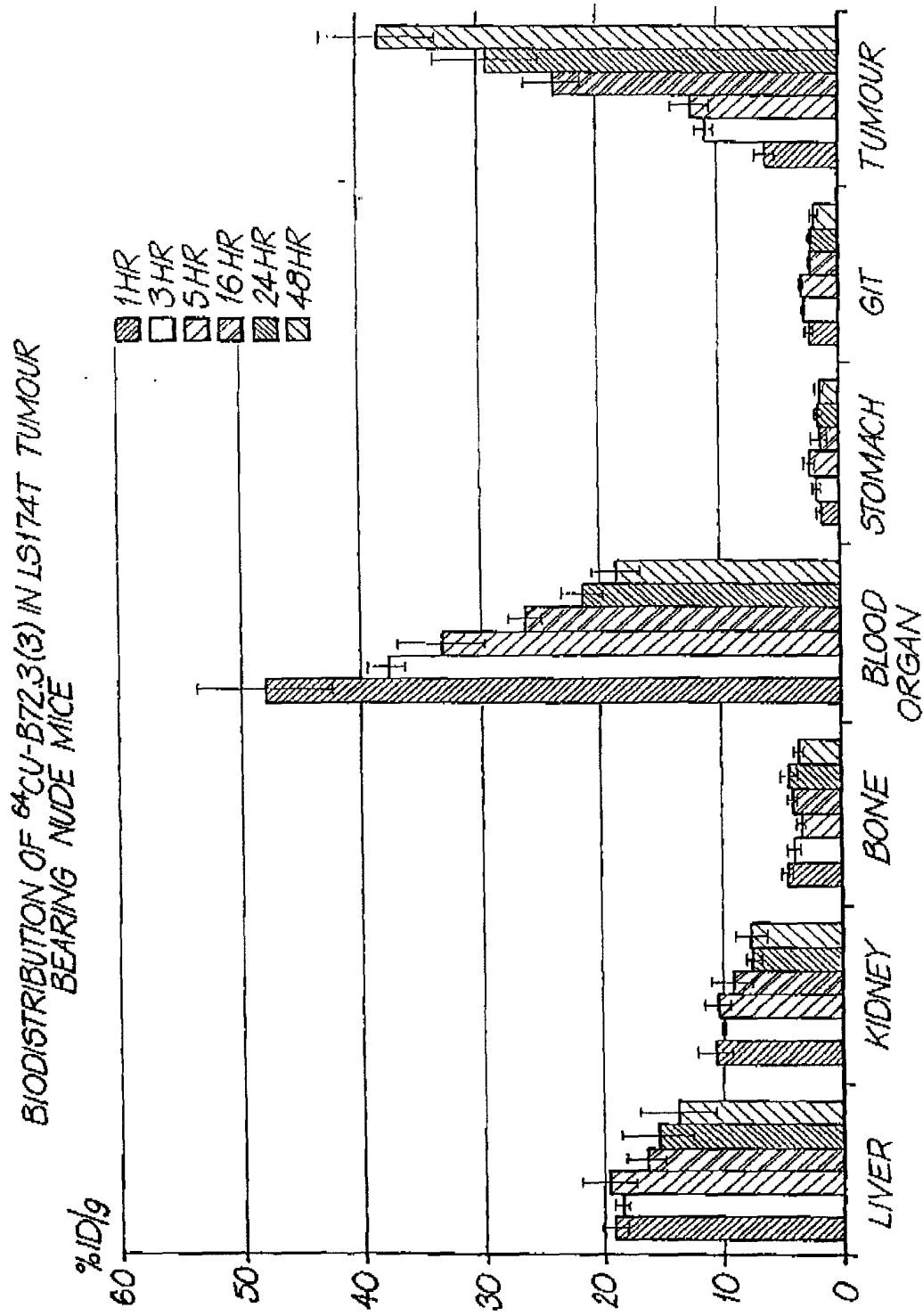


FIG. 6

TOTAL 4469860

WO 00/40585

PCT/AU00/00003

8/8

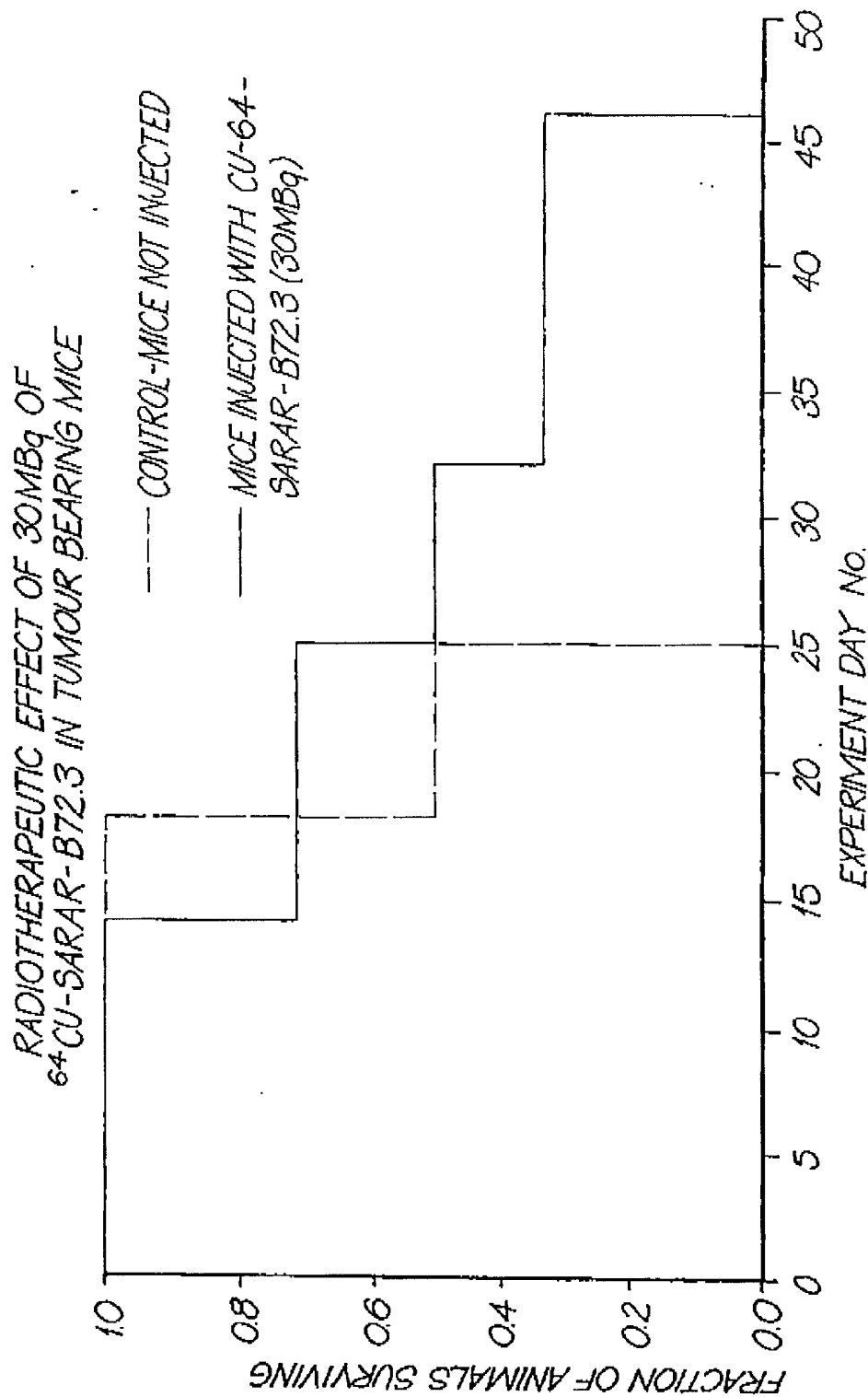


FIG. 7

JOINT DECLARATION FOR PATENT APPLICATION

As the below named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names;

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled

Cryptate Compounds and Methods for Diagnosis and Therapy, the specification of which:

- ☐ is attached hereto.
☒ was filed on July 5, 2001 as Application Serial Number 09/869,777 and was amended on _____ (if applicable).
☐ was filed under the Patent Cooperation Treaty (PCT) and accorded International Application No. _____, filed _____, and amended on _____ (if any).

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We hereby acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Prior Foreign Application(s)

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Country	Application No.	Date of Filing (day month year)	Date of Issue (day month year)	Priority Claimed Under 35 U.S.C. §119
PCT	PCT/AU00/00003	5 January 2000	n/a	n/a
Australia	PP 8038	5 January 1999	n/a	n/a

Prior United States Provisional Application(s)

We hereby claim priority benefits under Title 35, United States Code, §119(e)(1) of any U.S. provisional application listed below:

U.S. Provisional Application No.	Date of Filing (day month year)	Priority Claimed Under 35 U.S.C. §119(e)(1)

Prior United States Application(s)

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Date of Filing (Day, Month, Year)	Status X Patented, Pending, Abandoned

Power of Attorney

And we hereby appoint, both jointly and severally, as our attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith the following at attorneys and agents, their registration numbers being listed after their names:

ALTHERR, Robert F.	31,810	HANLON, Brian E.	40,449	NIEGOWSKI, James A.	28,331
BANNER, Donald W.	17,037	HEMMENDINGER, Lisa M.	42,653	PATEL, Binal J.	42,065
BANNER, Mark T.	29,888	HOLMES-SON, Michelle	147,660	PATHAK, Ajay S.	38,266
BANNER, Pamela I.	33,644	HONG, Patricia E.	34,373	PETERSON, Thomas L.	30,969
BECKER, Matthew P.	45,824	HOSCHET, Dale H.	19,090	POTENZA, Joseph M.	28,175
BECKETT, William W.	18,262	HYMEL, Lin J.	45,414	PRATT, Thomas K.	37,210
BERGHAMMER, Joseph J.	46,057	IRVING, Richard	38,499	RENK, Christopher J.	33,761
BODNER, Jordan	42,338	IWANICKI, John P.	34,628	RESIS, Robert H.	32,168
BOSS, Aimee M.	147,437	JACKSON, Thomas H.	29,808	RIVARD, Paul M.	43,446
BUROW, Scott A.	42,373	KAGAN, Sarah A.	32,141	RHODES, Christopher	47,022
CALLAHAN, James V.	20,095	KATZ, Robert S.	36,402	ROBINSON, Douglas W.	32,751
CHANG, Steve S.	42,402	KLEIN, William J.	43,719	RYU, Mike	38,604
CLUSE, Brian S.	47,497	LEE, David S.	38,222	SCHAD, Steven P.	32,550
COHAN, Gregory J.	40,959	LINEK, Ernest V.	29,822	SHIPLEY, Charles W.	28,042
COOPERMAN, Marc S.	34,143	MALONE, Dale A.	32,155	SHULL, Jason S.	47,085
CURTIN, Joseph P.	34,571	McDERMOTT, Peter D.	29,411	SKERPON, Joseph M.	29,864
DeMOOR, Laura J.	39,654	McKEE, Christopher L.	32,384	STECZYK, Joseph	34,442
ELPEL, Jeanne M.	41,456	McKIE, Edward F.	17,335	VAN ES, J. Pieter	37,746
EVANS, Thomas L.	35,805	MEDLOCK, Nina L.	29,673	WITCOFF, Sheldon W.	17,399
FEDORCHKO, Gary D.	35,509	MEECE, Timothy C.	38,553	WOLFFE, Franklin D.	19,724
FERGUSON, Catherine A.	40,877	MEEKER, Frederic M.	35,282	WOLFFE, Susan A.	33,568
FICKLER, Debra A.	46,699	MILLER, Charles L.	43,805	WRIGHT, Bradley C.	38,061
FISHER, William J.	32,133	MITRUS, Janice V.	43,808		
GARRETT, John R.	27,888	MOTTLEY, Darrell G.	42,912		
GLEBOCKI, Christopher R.	38,800	NELSON, Jon O.	24,566		

All correspondence and telephone communications should be addressed to:

Banner & Witcoff, Ltd.
Customer Number: 22908 (CHG)

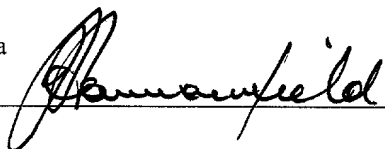
We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FIRST INVENTOR:

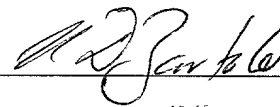
Full Name: SUZANNE V. SMITH
Citizenship: Australian
Residence: 279 Princes Highway, Sylvania, 2224, NSW, Australia.
Post Office Address: 279 Princes Highway, Sylvania, 2224, NSW, Australia.

Date: 13/ August 2007 Signature: Alux

SECOND INVENTOR:

200 Full Name: JOHN M. HARROWFIELDCitizenship: AustralianResidence: 19 Willcock Avenue, Daglish, W.A. 6008, AustraliaPost Office Address: 19 Willcock Avenue, Daglish, W.A. 6008, AustraliaDate: 8/7/01Signature: 

THIRD INVENTOR:

300 Full Name: NADINE M. DI BARTOLOCitizenship: AustralianResidence: 54 Glencoe Street, Sutherland, NSW 2232, AustraliaPost Office Address: 54 Glencoe Street, Sutherland, NSW 2232, AustraliaDate: 25/7/01Signature: 

FOURTH INVENTOR:

400 Full Name: ALAN MCLEOD SARGESONCitizenship: AustralianResidence: 53 Dunstan Street, Curtin, ACT 2605, AustraliaPost Office Address: 53 Dunstan Street, Curtin, ACT 2605, AustraliaDate: 31/7/01Signature: 